Imperial Institute
of
Agricultural Research, Pusa.

ANNALS OF BOTANY

EDITED BY

V. H. BLACKMAN, Sc.D., F.R.S.
PROFESSOR OF PLANT PHYSIOLOGY AND PATHOLOGY, IMPERIAL COLLEGE OF SCIENCE AND TECHNOLOGY, LONDON

AND

R. THAXTER, M.A., PH.D.
PROFESSOR OF CRYPTOGAMIC BOTANY IN HARVARD UNIVERSITY, CAMBRIDGE, MASS., U.S.A.

ASSISTED BY

D. H. SCOTT, M.A., LL.D., D.Sc., F.R.S.
LATELY HONORARY KEEPER OF THE JODRELL LABORATORY, ROYAL BOTANIC GARDENS, KEW

J. B. FARMER, M.A., LL.D., D.Sc., F.R.S.
PROFESSOR OF BOTANY, IMPERIAL COLLEGE OF SCIENCE AND TECHNOLOGY, LONDON

F. W. OLIVER, M.A., D.Sc., F.R.S.
QUAIN PROFESSOR OF BOTANY, UNIVERSITY COLLEGE, LONDON

AND OTHER BOTANISTS

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Cytology of Osmunda and Doodia.

I. On the Somatic and Meiotic Mitoses of Doodia.

BY

P. C. SARBADHIKARI, Ph.D., D.I.C.,

R.C.S. Scholar, Imperial College, London.

With Plates I–V.

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INTRODUCTION.

AN investigation on the cytology of *Osmunda* and *Doodia*, the results of which are here presented, was undertaken during the summer of 1920 with the idea of a critical study of the behaviour of chromosomes during both

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heterotype and homotype mitoses in the above two types. The cytology of *Osmunda* has been the subject of much study in the past. Twenty-three years have elapsed since the publication of Strasburger's (16) account of the heterotype division of *Osmunda regalis.* In the same year (1900) Wilson Smith (17) published an interesting account of the achromatic spindle in the spore mother-cells of *Osmunda regalis.* Prof. Farmer, with Moore (8), selected *Osmunda regalis* as one of the subjects dealt with in their memoir (1905) 'On the Meiotic Phase (Reduction Divisions) in Animals and Plants'. In 1907 Grégoire (12) described the origin of heterotype chromosomes in *Osmunda*, and maintained the lateral approximation of the chromosome of maternal and paternal origin during synapsis. In 1910 Yamanouchi (18) published his paper on *Osmunda cinnamomea*, where he also supported the parasynaptic theory of Grégoire.

Subsequently, Miss Digby's exhaustive study of *Osmunda* brought to light some interesting facts, hitherto unnoticed by previous investigators. Her paper 'On the Archesporial and Meiotic Mitoses of *Osmunda*' was published in 1919 (5), and her investigations leading to the conclusion 'that *Osmunda* affords strong evidence in favour of the telesynaptic theory of the origin of heterotype chromosomes' may be considered as forming a very important contribution to the literature of meiosis.

Being much impressed with the difference in the accounts given by various investigators, and with the divergent views expressed as to the origin of the heterotype chromosomes of the above fern, the present investigation was started. The main objective has been the comparative study of the two types *Osmunda* and *Doodia.* The present paper will be confined to the somatic and meiotic mitoses of *Doodia*, with special reference to the origin of heterotype chromosomes. *Doodia* is perhaps a more suitable fern than *Osmunda* for cytological study, owing to the fairly large size of the nuclei, the low number of chromosomes, and the clearness of figures. Sections of roots were examined for the study of somatic divisions.

METHODS.

Three species of *Doodia*—(i) *Doodia aspera*, R. Br.; (ii) *Doodia aspera multifida*, Hort.; (iii) *Doodia media*, R. Br., Australia and New Zealand—were minutely examined, and the root-tips of *Doodia aspera* proved particularly well fitted for the study of mitoses in the vegetative cells of plants. The cells are rather larger, and, in properly stained material, the cell-structures, especially the chromosomes, are to be seen with almost diagrammatic clearness.

The material used in this investigation has been grown at the Chelsea Physic Garden, under the direct supervision of the curator, Mr.

Hales, to whom I am greatly indebted for a constant supply of suitable material.

There was no difficulty in 'fixing' the root-tips, which in most cases proved to be very satisfactory; but considerable difficulty was encountered in fixing the sporangiferous apex of the fronds. Being much disappointed with the results, Professor Farmer's suggestion to Miss Digby of immersing the material in warm weak spirit before placing it in the different fixatives, was tried with very satisfactory results, and it rendered the ensuing fixation almost perfect. All the different strengths of warm spirit, ranging from 5 to 45 per cent., were tried, of which 35 per cent. gave the best result. The sporangiferous apices of the frond were cut, and, after plunging for a few seconds in 35 per cent. warm spirit, were placed in fixing fluids. A vacuum pump was used in all cases, and is essential to success in such work. Fixations were chiefly made between 11.30 a.m. and 1 p.m. on bright warm days.

The fixatives used were strong chromic, acetic alcohol, corrosive sublimate, Flemming's strong and Flemming's weak solutions, and Hermann's solution. The latter gave excellent results as the beaded nature of chromosome and the details of fission were most clearly brought out by this particular fixative. It afforded in this respect a distinct contrast with the effects of strong, as well as weak, Flemming's solution, where, in similar preparations, the chromosomes seemed to be more or less homogeneous. Some of the preparations of chromo-acetic acid were quite good where the material was kept in the fluid for about thirty-four hours with two or three changes, the material having been washed sixteen to twenty-two hours by means of rapid changes of water, gradually dehydrated and cleared in xylol. To 100 c.c. of chromic acid of 0·5 to 1 per cent. are added from one to four drops of glacial acetic acid—the proportion of acetic acid is quite sufficient to counteract any tendency to shrinkage due to the chromic acid. Merkel's solution proved to be unsatisfactory. All the materials were embedded in paraffin melting at 54° C.

Sections were cut at 4–10 μ thick, varying with the different stages sought. Most of the sections cut at 5–6 μ thick were quite useful for critical phases.

For staining the sections, Heidenhain's iron-alum-haematoxylin, both with and without a counter-stain, gave the best results, especially during the early prophases of heterotypic division. Some of the sections stained by Heidenhain's iron-haematoxylin, with orange G as counter-stain, turned out to be very successful for late telophases. For the spindle, and in some instances for the spireme, Flemming's triple stain was found to be preferable. Breinl (safranin, methylene blue, and orange G) was also used, though without much success.

SOMATIC AND PREMEIOTIC MITOSES.

Chromosomes in Late Telophase.

It is necessary to start the investigation at the late telophase, so that light may be thrown on the much-discussed phenomenon of the origin of heterotype chromosomes. Although the Telosynaptists and Parasynaptists are quite in accord with each others' views, in so far as the evolution of the somatic chromosomes are concerned, yet some clues might be obtained which would enable the heterotype chromosomes to be more easily traced. Of course, the 'resting nucleus' is not early enough to begin with. There is difficulty in unravelling the course of events, as some nuclei pass directly from somatic telophase into heterotype prophase, while others pass into interphase and subsequently to resting stage. The later phenomena is evident in the somatic mitoses.

The chromosomes break up into portions, and these are distributed throughout the nucleus. Each chromosome resolves itself into two parallel lines, which are sometimes homogeneous, but more often contain granules. Authors differ as to the period when longitudinal splitting is produced. Miss Digby (7) interprets the phenomena of telophase observed in *Osmunda regalis* as an example of longitudinal splitting of the premeiotic (somatic) chromosomes. The division commences sometimes at anaphase of the preceding mitoses, but it is clearly apparent when nuclear membrane is formed (Pl. I, Fig. 1). Several investigators have described and figured the vacuolization of chromosomes in plants, at this particular stage, in an almost diagrammatic manner. In our material, although the 'diagrammatic vacuolization' is not so clear as that of other workers (15), its presence is quite evident in rather late stages (Pl. I, Fig. 1). Some of the segments of chromosomes when examined in surface view are seen to be distinctly alveolized (Pl. I, Fig. 1). There is a clear median line, on each side of which are two parallel deeply staining lines (chromatin). This is especially apparent in sections fixed in Hermann's fluid.

The building up of the chromosomes, as in *Primula*, according to Miss Digby's account (4), is in the form of granules arranged in a simple row. The chromosomes are organized as beaded (uniseriate) filaments, and the same thing occurs in *Doodia* (Pl. I, Fig. 4). Gradually these granules, which at first only take the stain weakly, become more or less chromatic and end by forming a part of the filament, a homogeneous chord of smooth contour. According to some cytologists, these fine granules tend at first to gather themselves into small, definite, and paired masses; then these gradually disengage themselves; their substance is dispersed across a linin reticulum.

Grégoire (13) in 1906 showed that in the roots of *Allium* each chromosome resolves itself into two parallel lines composed of threads or granules. This parallelism is to be seen in parts of the linin, almost in a diagrammatic manner, in *Doodia* (Pl. I, Fig. 1). A clear space round the nucleus is visible when the linin framework is more or less distributed throughout the nucleus (Pl. I, Fig. 1). So the statement made by Grégoire (13), that in the *alveolization of chromosomes during the telophase the chromosome band dissolves itself into two parallel filaments with an intervening space*, applies in this case also. The investigators who describe at the telophase, or even metaphase and anaphase, a longitudinal splitting of chromosomes admit that these persist as double bands at the interphase and reappear in a distinct manner at the commencement of prophase. These double bands directly give rise by a close connexion of each half to definite chromosomes. These double structures are intimately associated in pairs, and do not lose their identity in somatic prophase after passing through the resting stage. Evidence of duality can be traced even at the resting stage, where some of the filaments are in pairs. This supports Miss Digby's statement (3) that in *Galtonia candicans* the relation between telophasic alveolization and longitudinal prophasic division is quite possible.

INTERPHASE.

The nuclear structure at the interphase is a network of almost diagrammatic regularity, composed of linin reticulum in which are embedded chromatic granules (Pl. I, Fig. 4). The nucleus presents certain parallel aspects which are present at the telophase. Thus, one recognizes a considerable number of parallel filaments which might be taken for double bands (Pl. I, Fig. 4) if one had not studied their origin. The parallelisms indicate that these chromosomes have hardly undergone any change of position since the commencement of telophase.

The nuclear structure is rather elongated, the filaments being long and passing one above the other. They are pale, with here and there knots deeply stained (Pl. I, Fig. 4). Numerous anastomoses can be seen. The whole nuclear cavity is occupied by the network, which does not leave any free space around the nucleolus in well-fixed nuclei (Pl. I, Fig. 4).

In *Osmunda cinnamomea*, Yamanouchi (18) describes an irregular reticulum, composed of small, highly-coloured, and jagged masses and slender filaments.

RESTING STAGE.

It is rather difficult to find out in quickly dividing cells of the root-tip any stage representing real 'rest'. Still, by careful observation, one may find some cells which are in so-called 'resting stage'. In the resting nucleus the chromatin is fairly evenly distributed throughout its substance, and there is a large nucleus (Pl. I, Fig. 3). Miss Digby had some difficulty in finding cells with real resting stage in *Galtonia candicans* (3); she describes 'resting nucleus' in the archesporial cells of *Osmunda regalis* (5) as composed of delicate reticulum, with filaments separated from each other and composed of granules. Beer (2) does not state definitely the constitution of the filaments, but he seems to admit the existence of independent chromatic granules.

Pl. I, Fig. 3 shows a very early stage of the so-called 'resting nucleus', where it is provided with delicate network in which are distributed small chromatin granules which are quite distinct and take a deep stain in Heidenhain's iron-alum-haematoxylin.

The threads in most of the nuclei are so delicate and rare that the nucleolus appears to lie in the centre of a perfectly colourless space. The cytoplasm with fine granules is of uniform network.

One or more nucleoli can be found at this stage as well as in prophase. In some cells several nucleoli may be aggregated in a peculiar manner, and these by division assume a peculiar hour-glass form (Pl. I, Fig. 2). This phenomenon has been observed and described in animals and in plants by several investigators.

PROPHASE (SOMATIC AND PREMEIOTIC MITOSES).

In the very early stage of prophase the reticulum is seen to break up in certain parts (Pl. I, Fig. 5). The material of each reticulate unit is represented at the beginning of prophase by a double or single row of granules which unite afterwards with one another (Pl. I, Fig. 4). The fine granules tend, at first, to gather themselves into small, definite, and paired masses; then these gradually disengage themselves and take their place at the points of intersection of the filaments. After condensation of reticulate unit, the zigzag threads are formed which soon begin to straighten out (Pl. I, Fig. 6), giving rise to definite chromosomes.

It is possible for the separated portions of the reticulum to be condensed directly into slender threads, representing definite chromosomes, without passing through the zigzag stage. Miss Digby (3) describes a similar occurrence in *Galtonia*.

Some investigators are of opinion that at the commencement of prophase the chromosomic element presents the form of alveolized bands which become progressively concentrated while retaining their thickness. They gradually lose their alveolized form in which longitudinal division is slowly produced, or these bands give rise by irregular repartition of their substance to a zigzag filament which undergoes a longitudinal division before becoming a definite chromosome. Grégoire (13) in *Allium* maintains the condensation of separated portions of the reticulum directly into slender threads without passing through a zigzag stage.

The alveolized bands, giving rise to definite chromosomes, are identical with those of the preceding telophase, and after being indistinguishable, though not really lost, in the network of the resting stage, disengage themselves afresh at the commencement of division. They would thus give rise, by a method of gradual concentration, to chromosomes.

Perhaps it will not be quite out of place to quote here the description given by Farmer and Moore (8) regarding the like phenomenon in the somatic cells of *Periplaneta americana*:

> '. . . the cells of the premeiotic series which are about to divide, whether they are encountered within the sexual glands or elsewhere in the tissues of the body, present the rather characteristic appearance represented in fig. 40, a very irregular network of chromatin and linin being grouped within the nuclear membrane round one or two highly chromatic nucleoli. . . . At first, the cells which are preparing for division present an almost even granulation of the chromatin within their nuclei, and this in its consistency strongly suggests a foam structure of the ordinary type; but after a time the "chromatic confusion", as it were, sorts itself out into obvious condensations or cloudy areas, and it is apparently unquestionable that each of these primitive chromatic clouds is individually the forerunner of one of the future chromosomes. The gradual condensation which occurs in each such cloud proceeds, moreover, in such a manner that the chromatic granules become arranged or grouped into two distinct rows or tracts. So that by the time the individual chromosomes have attained to some sharpness of definition they appear also as if they had been split longitudinally from end to end.'

Grégoire, as long ago as 1906 (13), as the result of a careful investigation of *Allium* and certain other Liliaceae, concluded that the 'vacuolization' which occurs in the telophase of a mitosis is responsible for the appearances which have been interpreted by some authors as signifying longitudinal fission.

Up to the publication of Miss Digby's (5) work on *Osmunda regalis*, the descriptions which have been given of the somatic mitoses of Pteridophytes by different authors include an identical process in the stage or prophase. Yamanouchi (18), however, found in *Osmunda cinnamomea* that, contrary to the general rule in plants, *prophasic bands did not succeed the alveolized bands of the telophase.* Indeed, from Pl. I, Fig. 4 one recognizes in the nuclei at the commencement of prophase the presence of alveolized bands. Then appears a stage of zigzag filaments (Pl. I, Fig. 5). The prophase constitutes a series the reverse to that of telophase. The chromosomic longitudinal halves, which have been separated at the end of the telophase, come together and form dual filaments. The two filaments may be still granular and separated; then they become intimately associated and form a single thread which will be segmented into chromosomes. Fine anastomoses bind together the different chromatic filaments.

A remarkable and somewhat complex growth of chromatin within a matrix, which is apparently the linin of other authors, has been described by P. Martens (13*a*). The account suggests an independent growth of a thread of 'chromonématique' substance, the latter apparently identical with a thread of nuclein material, embedded in the achromatic linin. By a series of convolutions in the former, a flat ribbon is formed, and owing to the thinning out of the middle portions lying between the edges of the ribbon-like convoluted band of 'chromonématique' substance a separation into two longitudinal halves occurs in the chromosome *anlage*. This account, in effect, would seem to imply the disposition, *on the two sides of the ribbon*, of chromatin particles that originally were arranged *lengthwise* in the 'chromonématique' filament. Martens (13*a*) believes his observations dispose of both the 'alveolization' and the 'longitudinal fission' theories of chromosome division, in so far as telophase and (presumably) prophase are concerned.

LONGITUDINAL SPLITTING OF THE CHROMOSOMES.

Pl. I, Fig. 1 shows that an indication of vacuoles can be observed along the axis, which develop into more or less continuous thread. The nature of the double spireme is to be seen from the earliest stage. At first it consists of a double series of granules which place themselves on the nuclear framework, side by side. The dissociation (separation) of the spireme into two distinct longitudinal bands is considered as a definite fission; Grégoire (12) is of opinion that the longitudinal division is essentially a prophasic phenomena. Sharp (15) also, in describing the somatic chromosomes in *Vicia*, is of opinion that the longitudinal splitting is a phenomenon of prophasic period. Although these investigators agree in ascribing the process to a fairly early stage, they nevertheless differ concerning the *precise moment when the splitting is produced*. On the one hand, according to several authors, division is only effected at the moment when the chromosomes are inserted into spindle fibres, or indeed when they are definitely arranged in the equatorial plate. But this view regarding the somatic chromosomes is not accepted by Grégoire (13). On the other is the opinion, generally admitted, that integral bipartition of the elements of chromosomes occurs by means of a series of vacuoles being formed along the axis. These vacuoles, accordingly, would unite to form a continuous split separating the sister halves of the same chromosome.

METAPHASE (SOMATIC AND PREMEIOTIC).

The chromosomes at the metaphase, at least those of central parts, are laid down parallel with spindle fibres, and present their nearest extremities, often a little curved (Pl. I, Fig. 7), arranged very regularly in the equatorial plane; the rest of the body is more or less straight or sometimes curved (Pl. I, Fig. 7). The chromosomes of the edges were nearly always disposed according to their length in the equatorial plane, and form a sort of crown which is rather distinct in the metaphases, seen from the pole (Pl. I, Fig. 7).

The double nature of the chromosomes is remarkably prominent when they arrange themselves upon the spindle just before their anaphasic separation. In this particular stage, with special reference to somatic chromosomes, the longitudinal fission is so distinct and well extended that they seem to separate the daughter chromosomes from one end to the other (Pl. I, Fig. 7). The longitudinal split is extremely perfect.

At first, the chromosomes are attached to the spindle by one end and lie at right angles to the plane of the spindle (Pl. I, Fig. 7). As the chromosomes take their place on the spindle, the spindle fibres are attached to them on particular parts. The point of attachment of the rather long chromosomes is distinctly at the middle (Pl. I, Fig. 7).

Some of the chromosomes, instead of remaining straight, become somewhat twisted in the form of a more or less wider loop or V, the sides of which are well extended (Pl. I, Fig. 8). The split appears to be more prominent in the straight chromosomes than in the twisted ones.

It is necessary to call attention to a rather curious and interesting phenomena observed in metaphase. At present we only mention it, as we intend to deal with it later. Some of the chromosomes in metaphase were observed in pairs and appear to cross each other (Pl. I, Figs. 8, 9, 10, 11) in a way which recalls what is known as chiasmatype. The appearance in question would evidently have presented less difficulty, and less interest perhaps, had it been found in the heterotype instead of in somatic mitoses.

Gates (10), in his study of the chromosomes in somatic mitoses in *Oenothera*, mentions the paired arrangement of chromosomes which is so conspicuous in metaphase.

The nucleolus vanishes with amazing rapidity and leaves practically no evidence of its former existence, even at such an early stage of metaphase, when chromosomes complete their equatorial arrangement.

ANAPHASE.

The anaphases of somatic mitoses are represented in Pl. I, Figs. 12, 13, 14. Fig. 12 shows a rather earlier stage of anaphase, in which the chromosomes, in the form of loops or rods, are on their way to the poles; the longitudinal fission in some of the chromosomes is still quite evident. In some the split is almost through and through, as was seen in metaphase, whilst in others it is visible only in some portions (Fig. 12). Fig. 13 shows a later stage where a more compact group of the daughter chromosomes is being formed. Gradually the chromosomes approximate closely to form a confused and more or less homogeneous mass. In Fig. 14 is found a still more advanced stage, in which the chromosomes are fused into masses in the middle of which vacuoles are formed. In the median plane of the spindle are found indications of a cell-plate.

GENERAL CONSIDERATIONS ON THE SOMATIC (PREMEIOTIC) MITOSES.

The points of special importance emerging from the above events are:

1. The persistence of longitudinal fission (which is most marked in metaphase) in the succeeding stages. The longitudinal fission seems to be of telophasic origin.
2. All the phenomena in connexion with the telophase of previous division have an important bearing on the phenomena of early prophase.
3. The chromosomes, the sides of which separate widely, become alveolized distinctly in telophase, although the first indication may be observed in rather advanced stages of anaphase.
4. The formation of each ultimate chromosome involves a side-to-side approximation of the parallel longitudinal halves which have originated during the preceding telophase.
5. Some variation of nucleolus in the form of hour-glass shape was observed.
6. In metaphase, some of the chromosomes were observed in pairs.

FIRST MEIOTIC DIVISION.

In the following paragraphs no attempt will be made to discuss in any detail the vast literature of the much-debated question regarding the chromosome reduction. Cytologists are familiar with the two different accounts concerning the mode of origin of heterotype chromosomes. In this paper an attempt will be made to state some of the broad, rather clear, and well-defined aspects revealed by the microscope, with special reference to presynaptic and second contraction figures, and consequently the possible interpretation derived from the evidence of this particular specimen.

PRESYNAPTIC AND SYNAPTIC PHASES.

In studying the prophase of first meiotic division, one is confronted at the outset with some of the striking resemblances which are found in the later telophase of the somatic chromosomes. The same alveolar or reticulate structure, with its familiar arrangement of beads in single or double lines, can be seen in such a distinct manner as to suggest a sequence of events between the telophasic alveolization of somatic chromosomes and the vacuolated nature of the early heterotype chromosomes.

As in the somatic divisions, the chromatin of the very early prophasic nucleus, or at least those which are destined to proceed to heterotypic mitoses, seems to be diffused throughout the linin and to stain more faintly. The granules of chromatin seem to be distributed irregularly; their aggregation can be seen more in the angles where the delicate filaments, or alveoli, meet (Pl. I, Fig. 15; Pl. II, Figs. 16, 17). These figures show cells where the reticulum is distributed rather uniformly throughout the nuclear cavity; the beaded linin reticulum seems to be more or less of coarse nature; careful focus will reveal also some of the delicate threads of reticulum running in pairs (Pl. II, Figs. 17 and 18). These phenomena become clearer and clearer as we gradually proceed to the comparatively later stages. The reticulum gradually loses its reticulate character and at the same time the chromatin of the beads becomes more and more uniform until they show a distinct spireme nature (Pl. II, Figs. 20, 21). Figs. 18 and 19 show a slightly more advanced stage where the linin appears to withdraw a little from the centre to the framework, leaving a more or less clear space in the centre of the nucleus. Some of the linin fragments are seen to be vacuolized, and the pairings of the threads, forming distinct parallel lines, are evident from Pl. II, Fig. 19. In still more advanced stages, where the linin fragments assume more definitely their usual spireme character, long lengths may be seen running parallel to one another (Pl. II, Figs. 20, 21). The parallel arrangement is seen to be traversed, in some places, by beads connecting the two adjacent filaments (Pl. II, Figs. 19, 20) and giving an appearance of a sort of ladder-like arrangement.

First Contraction.

Just before the first contraction the spireme threads withdraw from the periphery and aggregate in the centre irregularly (Pl. II, Fig. 22). The nuclear periphery is quite clear and the threads are aggregated towards the centre (Pl. II, Fig. 22). The spireme threads exactly resemble a loosely tangled skein of wool. Fission is almost lost at this stage, traces of it being visible only in portions of the thread (Pl. II, Fig. 22). At this stage one is baffled in the attempt to trace out the continuity of the thread. Subsequent attempt might lead to the conclusion that the continuous thread is present. The falling back in loops is one of the characteristic features of this stage, from which some arrive at a positive conclusion regarding the continuity of threads. The big spherical nucleolus is almost hidden in the extremely complicated tangled mass.

Synapsis.

We have mentioned that the above stage, namely, withdrawal of spireme threads from the nuclear periphery, is of short duration. Following this, the stage of synapsis proper begins. The threads or the coils of the linin are aggregated densely and occupy their position at one side of the nuclear periphery (Pl. II, Figs. 23, 24). Gradually almost all the contents of the nuclear cavity are aggregated so as to form a knot; this ball of tangled threads lies at one side of the nucleus; some parts of the tangled thread remain distinctly free from the general mass and twist and loop over each other (Pl. II, Fig. 24). One can recognize from this bending over of the loops the presence of a longitudinal split which is temporarily lost in the tangled thread (Pls. II, III, Figs. 24, 28). Most of the outer bends of the loops of spiremes in presynaptic stages are seen to run parallel in parts in such a way that they suggest nothing but close approximation of the two threads of the same filament (Pl. II, Fig. 25). When these parallel threads enter the synaptic stage they usually condense and contract and gradually approximate in such a way that they appear almost of homogeneous structure (Pl. III, Figs. 28, 29). In most of the favourable preparations the presence of the fission, at least in certain parts, is never lost. One must be very careful in the choice of fixatives, especially at this particular stage. Hermann's solution is extremely suitable at and from this particular stage to demonstrate the beaded nature of the spireme, which shows up very clearly (Pl. II, Figs. 23, 24, 25). When fixed in Flemming's weak and strong solutions the spiremes appear as rather homogeneous strands (Pl. II, Figs. 26, 27). The spherical nucleolus, which only stains feebly, is situated excentrically at the clear nuclear cavity (Pl. II, Figs. 23, 24).

The process of the evolution of the synaptic thread and the relation of the subsequent duplicate character are extremely interesting in *Doodia*. Out of the densely staining intricate mass, which sometimes makes it difficult to follow the sequence of events, can be seen the association of threads of each monovalent chromosome. That the threads have undergone a process of complete sorting out in synapsis is evident from the fact that *as the spireme emerges from synapsis the difference in thickness of the filaments is most remarkable.* The delicate string-like filaments now assume the form of a ribbon with longitudinal splittings more and more prominent. The same appearance of the spireme as was seen just after the first contraction stage, or, in other words, at the beginning of synapsis, is again met with, the only difference being the thickness of the spireme forming a ribbon-like structure. The less intricate anastomosed ribbons are thrown into loops and some of them extend quite to the nuclear periphery. One can recognize from these the distinct pairings at intervals in the broad ribbons (Pl. III, Fig. 28) without difficulty. The association of the threads is evident enough from the sides of the loops. There cannot be any doubt, even from this phenomenon (and the subsequent event), that the association of the threads is now almost complete, and that the univalent chromosomes consist of two threads. From this stage onwards, especially just before the second contraction stage, the univalent spireme will be seen to be distinctly of a double nature—the doubleness is due to the close proximation of two threads.

The interesting results which have been published by Gates (11) as the result of his investigations on hybrid Oenotheras lead one to the belief that there also the doubling is in reality due to a split which closes up later, rather than to the association of separate spiremes.

Hollow Spireme.

As the synaptic knot loosens, the nuclear cavity is again invaded by the irregular masses of threads, provided with beads of chromatin in single or in double rows (Pl. III, Fig. 29). At first the split is represented by the beads of chromatin which lengthen out and arrange in rows. The beads are joined by very fine threads. Gradually in more advanced stages the halves of each beaded line separate from each other and run in two distinct parallel rows. Again the excellent results obtained from Hermann-fixed materials at this stage deserve mention. Pl. III, Fig. 30 shows a carefully fixed section where every portion of the spireme thread distinctly shows its split character (fission). The fission is not complete, but is closed up at regular intervals, especially at the earlier stage of hollow spireme. The sides of each thread of the univalent spireme become more or less homogeneous as they proceed to the advanced stage and the separation in each thread becomes increasingly wider. Some of the split univalent spiremes may present the appearance of chain-like structure, while others show a sort of moniliform aspect. These graded swellings cannot be the approximation of originally separate filaments, nor can they be a lateral conjunction of two distinct entire spireme filaments, representing each a somatic chromosome, as Grégoire (12) described them, but they clearly represent separation of univalent chromosomes into two distinct half-threads. *They are not the union of slender filaments in pairs, but the disunion of two threads of the same filament (the univalent spireme).* Although they may appear in some parts to be formed of two interlaced filaments coming together, in reality they are two dissociated threads separating from one another. The point will be more clear as one proceeds to the later stages. Pl. III, Fig. 31 depicts a rather advanced phase of hollow spireme, where some of the univalent spiremes with more pronounced fission show a tendency to fall over in loops. Some of the filaments (especially that marked *a*) show clearly that the fission goes right along from one end to the other, and the striking similarity with the fission of somatic chromosomes (Pl. I, Fig. 7) leads us to the conclusion that *they are, like somatic chromosomes, sister halves of the same chromosomes and not approximation of two entire somatic chromosomes.*

The existence of free bifid ends can be maintained in this late stage (Pl. III, Fig. 31).

Miss Digby (5) describes in *Osmunda* how the fissions become rapidly closed up as they proceed to still later stages; subsequently, just before the second contraction, there cannot be seen a single univalent chromosome where fission is visible. In her Fig. 59 (*Osmunda*) one can see that there is not even a trace of fission in any of the spiremes, and they all look quite homogeneous. We find that our material differs in this respect. In *Doodia* we have not been able to find a single instance where this complete closing up of the fission can be observed. From the evidence afforded by this material we are of opinion that the fission is never closed up; its presence can always be recognized—although it becomes more or less indistinct in the thick spireme stage. It is not denied that the separated halves of the filaments gradually re-approximate, that this process continues to the later stages till they are very indistinct, and that there are stages where their presence is extremely difficult to recognize. But, nevertheless, we cannot say for *Doodia* that the split ever *becomes absolutely closed up*, to reappear after some time. The halves become more distinct, but not after the closing up *altogether*.

After a considerable amount of loosening of the synaptic knot, and the nuclear cavity being filled up by the beaded univalent spiremes, the first indication of 'looping over' of the spiremes is evident (Pl. III, Fig. 31). They loop over and twist in certain places and then run parallel for some distance (Pl. III, Figs. 30, 31). Gradually, in a little advanced stage, the univalent spiremes are seen to run parallel very closely and the space between them becomes less and less (Pl. III, Fig. 32). Pl. III, Fig. 31 shows a 'looping over' of a spireme, which is beaded in character, emerging from the big nucleolus at the periphery. The sides of the loops with fission, attached to the nucleolus, are seen to run parallel with each other. Sometimes just the reverse phenomena happens, where the spiremes, after running parallel for a considerable portion in the nucleolar cavity, suddenly take a twist and turn in opposite directions (Pl. III, Fig. 35). As the twisting, looping, and running in parallel of the two univalent spiremes happens, their sides seem to be more thickened and condensed (Pl. III, Figs. 33, 34, 35). Subsequently, just before the second contraction, the nuclear cavity is traversed by the thick spiremes, rather densely aggregated, more in the centre than in the periphery (Pl. III, Fig. 36). It is here only that we notice the gradual obscuring of the splitting of univalent spiremes, but, nevertheless, their presence can be recognized in some, especially those which are situated at the periphery. Out of the rather intricate anastomoses of the spiremes, some are seen to be in close contact with each other (Pl. III, Fig. 36), but the fine connecting strands which were described and figured in *Osmunda* by Miss Digby (5) are not found in this material. The univalent spiremes assume various shapes in the form of S or U (Pl. III, Figs. 31, 32) or V or in rings, or in the form of two coiled strings, as they prepare for combination into bivalent chromosomes. The approximation of two threads of univalent spiremes has a remarkable analogy with the conjunction of two univalent spiremes to form a bivalent chromosome. This statement will be more clear as we proceed to the later stages.

Regarding the arrangement of the univalent lengths of spireme there is no hard and fast rule. The spiremes, when they are transversely segmented, show at the beginning an end-to-end arrangement (Pl. III, Figs. 33, 34, 35); but later on they may be joined side by side (Pl. III, Fig. 36). If we really grasp the meaning of the two terms parasynaptic and telosynaptic, which are in so constant use by American cytologists, the difference, as Prof. Farmer (6) has already shown, is not one of any fundamental importance, and we might say that the telosynaptic arrangement in the synaptic stage, the duration of which is unusually long in the heterotype mitoses, may become distinctly parasynaptic in late post-synaptic stage. In other words, at a late stage after the second contraction, when the two univalent spiremes become closely appressed to form a bivalent chromosome, the arrangement is often 'parasynaptic'.

THICK SPIREME AND SECOND CONTRACTION STAGE.

At the termination of the hollow spireme stage, and just before the advent of the second contraction, the filaments in the nuclear cavity thicken and become entangled in the centre; gradually, as the drawing in of the filaments from the periphery proceeds, the looping over towards the centre becomes more and more apparent (Pl. III, Fig. 37). Their attachment to the nucleolus shows that some of them are transversely segmented, and these segmented portions are proceeding to conjoin with each other (Pl. III, Fig. 37). These conjunctions are also evident, although rare, in the rather confused, thick, entangled mass in the centre, from which *some of the portions of loops escape, showing distinctly their parallel univalent nature* (Pl. IV, Fig. 39, more especially towards the periphery. Then, after loosening of these thick filaments with their outstretched peripheral loops, the nuclear cavity is seen to be more or less invaded by the conjoining spiremes (Pl. IV, Figs. 40, 43, 44). In some the two univalent filaments, with their fission still visible, run almost parallel to each other (Pl. IV, Fig. 44). Others, after running parallel only for a short distance, become closely appressed to each other, showing bivalent character (Pl. IV, Fig. 44). Others are conjoined in such a way as to present the structure of a ring (Pl. IV, Fig. 43), whilst the conjunction of others is somewhat irregular (Pl. IV, Fig. 43). At any rate some indication of conjunction, in one form or another, can be well recognized in these stages (Pls. III, IV, Figs. 37, 38, 39, 40, 43, 44). Gradually these conjunctions are shown in a pronounced way by the more obvious bending over of the loops, where the two arms of the loops become so closely appressed that their bivalent nature cannot be denied (Pl. IV, Fig.

47). It is from this stage that the original fission of each univalent spireme becomes extremely obscure and seems to be almost lost. But in the filaments which keep themselves aloof, so to say, from participating in the process of coalescence to form bivalents one cannot miss the presence of fission (Pl. IV, Figs. 39, 40). The two univalents are, however, closely appressed, and they seldom lose their line of union, which represents the line of future separation of the two elements of each bivalent (Pl. IV, Figs. 42, 43, 44). The splitting of the bivalents might be said to bear a close analogy with the fission of univalents. The qualities with which we are confronted here are only in reality the result of close conjunction of two univalent spiremes, and not the result of renewed disjunction into two threads of the thick spireme, as Grégoire (12), Yamanouchi (18), &c., describe them in *Osmunda*. Several details in some of our figures (Pl. IV, Figs. 39, 40, 43, 44) are of such a nature as to give support to this interpretation. In the first place, Figs. 39, 40, 43, and 44 clearly reveal that the interlaced branches of the spiremes are nothing but the close approximation of two filaments. In the second place, Pl. IV, Figs. 41 to 47 show the bivalent chromosomes are the result of the looping and bending over considerably of the univalents.

In short, the analogy of early and late heterotype stage remains identical throughout this evolution—the same general aspect, the same interlacement of filaments. The doubleness of heterotype is identically the same as in somatic chromosomes, which is due to a longitudinal splitting. In the late heterotype prophase, or, more clearly, in the second contraction stage, the phenomena of conjunction of entire univalent chromosomes in pairs may be called a repetition of the process which one finds in early prophase, where the association of the threads in pairs to form univalent chromosomes takes place.

Pl. III, Fig. 38 shows a nucleus where second contraction is not quite so accentuated and is apparently very quickly passed through, judging from working through of spore mother-cells. Some of the thick spiremes in the periphery (occupying the opposite extremity from the mass) show their original univalent structure, which is suggestive enough that they did not take any part in the process of conjunction (Pl. III, Fig. 38). Of course, in second contraction the process of conjunction of two univalent chromosomes takes place; but it does not necessarily follow that each one of the spiremes should take part in the above process. Isolated instances to support this statement are not rare (Pl. III, Fig. 38). Although this process of pairing of univalent chromosomes is rather obscure in the knotty conglomeration of the second contraction stage, it is quite evident from the subsequent loosening of second contraction (Pl. IV, Fig. 39) that this process does happen. Pl. IV, Fig. 40 shows that some of the bivalent segments have come out of second contraction, and give evidence both of *fission and conjunction*. The thick spiremes, which consist of the two univalents and which take rather deep stain, are segmented transversely, and show an end-to-end arrangement (Pl. IV, Figs. 45, 46). The thick spiremes, which are the product of the conjunction in pairs of univalents as they come out after loosening of second contraction, are seen in the nuclear cavity in various forms. At first, the majority of them are seen in loops, emerging out of the entangled mass and showing their bivalent nature, although their constituent parts (i. e. the two univalents with their original fission) are not lost (Pl. IV, Figs. 45, ch. A, B, C). Some are arranged in the form of a cross (Pl. IV, Figs. 42, 46). Others, although rare, may assume the form of a ring, where the constituent parts (i. e. the two univalents with original split) are entirely lost (Fig. 45, ch. D). Farmer and Moore (8) describe this ring-shaped structure as due to the approximation and fusion of extremities previously free from one another. Others may be arranged like two coiling springs while retaining their univalent character (Pl. IV, Fig. 46, ch. A). Some others, which are more or less situated near the periphery, are semilunar in appearance (Pl. IV, Fig. 46), while still others are quite straight and rod-like in structure (Pl. IV, Fig. 45). All these show, more or less, their univalent combination, or, in other words, they show still more definite bivalent chromosomes, many of them demonstrating *fission in their univalents*, somewhat concentrated. But on proceeding to later stages one finds that these bivalents are so closely appressed to each other that their univalent combination is almost lost, the splitting becomes more and more obscure (Pl. IV, Figs. 42, 49). In Fig. 42, ch. A represents a bivalent ribbon-like chromosome, with one end flattened, and a rather prominent splitting bears a close analogy with a somatic chromosome. The univalent nature, in some of them, can still be traced out, although with difficulty, from a rather indistinct arrangement of beads. Gradually, with their progressive evolution, the splitting is entirely closed up for some time, where the segments appear to be very thick and take deep stain (Pls. IV, V, Figs. 49, 50). Each side of the longitudinal splitting represents a univalent chromosome. The question whether this split is entirely closed up for some time and then reappears, or whether after complete conjunction (of univalents) the bivalents as they come out of a second contraction undergo a longitudinal splitting, appears to be involved in obscurity. At any rate, these splits become reduced and condensation is so great that the *bivalent chromosomes look like large black masses* (Pl. V, Fig. 50). Before diakinesis these very large bivalent chromosomes, which stain deeply, become much contracted and are scattered all over the nuclear cavity (Pls. IV, V, Figs. 49, 50).

DIAKINESIS.

The familiar figure in diakinesis is characterized by thick, densely stained, rod-like segments of bivalent chromosomes, arranged in pairs (Pls. IV, V, Figs. 49, 50) throughout the nucleus. It does not necessarily follow that all of them should be in pairs; for it is quite possible at this stage that each of the monovalent chromosomes of many of the bivalent groups can be almost or completely separated from each other. We have already noted that the bivalent chromosomes assume various forms. Some are arranged in the form of crosses (Pl. V, Fig. 50), while others present the appearance of rings (Pl. IV, Fig. 49); Y-shaped, V-shaped, and W-shaped forms are also familiar (Pls. IV, V, Figs. 49, 50), and simple, small, rod-like structures can also be observed (Pls. IV, V, Figs. 48, 49, 50). Gradually, as the monovalent constituents of each bivalent chromosome become detached from each other, they appear to be thickened and lose their individual characteristic shape. They become condensed and thickened and take dense stain, with the result that their individuality can hardly be detected. Although some of the bivalents separate into two monovalents, yet immediately after this they appear to unite again, at the time of the formation of the spindle fibres (Pl. V, Fig. 51).

METAPHASE.

At the completion of the spindle formation the chromosomes are seen to be aggregated on the equatorial plate in a more or less definite order (Pl. V, Fig. 51). The chromosomes at this stage seem to be more or less oval-shaped; in some the extremity of one side is very much pointed (Pl. V, Fig. 51). Some are of rod shape, and subsequently divide transversely across the middle. The longitudinal splitting appears so obscure at this particular stage in our material, that the point of union looks like a homogeneous mass (Pl. V, Fig. 51). Certainly in later stages their presence can be recognized, where the cleavage becomes more and more apparent, and subsequently the univalent component of each bivalent combination becomes separated from the other. It is from here that the process of disjunction begins, and the dissociation of the paired approximated halves is finally completed in a later stages.

ANAPHASE.

During anaphase the chromosomes, at first, seem to be extremely condensed, and crowded in such a way that the individual members are extremely difficult to distinguish. Gradually, from the condensed mass, fragments come out which soon become alveolized (Pl. V, Fig. 53). During the process of alveolization of chromosomes a cell-plate is formed in the spindle and the chromosomes are seen to be in more advanced stage.

TELOPHASE.

The longitudinal splitting of each daughter chromosome becomes more and more accentuated, and finally gives rise to the vacuolated appearance

(Pl. V, Figs. 54, 55). This phenomenon is identical with and bears a striking resemblance to the late telophase of a somatic chromosome. The same threads, which are the product of fragmentation of the alveolized bands of daughter chromosomes, run parallel to each other (Pl. V, Figs. 54, 55). The same beaded nature, either arranged in single or in double lines, is also evident in heterotype telophase. The splitting of the daughter chromosomes into longitudinal halves, which will be reassociated in the succeeding prophase, has a remarkable affinity with the splitting and alveolization of somatic telophase.

HOMOTYPE MITOSIS.

The events of the homotype mitosis are so difficult to follow in detail that at present it will not be possible for us to attempt a discussion of this division. The majority of the chromosomes become so much thickened and aggregated that their real form is difficult to identify. The swellings of chromosomes are such as to make it impossible to trace out their separate individual structures. Subsequently, as they pass to telophasic events, the structure of chromosomes, which is obscured by much beading and alveolization, presents a remarkable affinity to heterotype and somatic telophase. Each nucleus of the tetrad is seen to be uniformly distributed by the same alveolized bands, just in the same way as in somatic and first meiotic division (Pl. V, Fig. 56). The alveolized bands subsequently break up and the longitudinal halves of beaded structure resemble those of somatic and heterotypic telophase. The persistence of longitudinal fission is suggestive enough from the presence of double arrangement of chromatin granules which can be observed at intervals (Pl. V, Fig. 56).

GENERAL CONCLUSIONS.

It has already been mentioned that at present the object of this account of *Doodia* is to attempt to follow out some of the broad mitotic phenomena, and to suggest a possible explanation of the observed sequence of events, with special reference to this particular plant. Generalization and undue reliance on a single type, however promising it may be for investigation, is indeed the origin of many misconceptions in this particular branch of study. Such being the case, any conclusions arrived at must be considered in the first place as only holding good for the particular species investigated.

Those who have worked on these problems are in a position to realize the special difficulties that one has to encounter in dealing with any one particular type. In this case, the difficulty is due to the extreme rapidity with which certain phases of nuclear division are passed through. Attempts have been made to show in Pl. V, Fig. 57 how difficult it is to interpret the chromosomic evolution just because of this rapid progress. Pl. V, Fig. 57 shows three adjacent sporangia where three widely divergent stages are visible. In the figure sporangium A shows most of the nuclei in synapsis, while sporangium B shows them in second contraction stage. In sporangium C one can see late telophase of heterotype division where the chromosomes are much alveolized. In some sporangia, nuclei with different stages were found. In cases like these one is easily liable to misinterpret the true sequence of chromosomic evolution.

As a result of a prolonged study of this particular fern, extending over a period of several years, we may be permitted to say that every precaution has been taken in our attempt to prevent overlooking the important phases throughout the chromosomic evolution, with special reference to *synaptic and second contraction stages.*

Before arriving at a definite conclusion, after correlating some of our facts with those of other workers in this line, we think it desirable to give a brief review of the preceding details emerging from this investigation. However conflicting the opinions may be with regard to the origin of chromatin threads, it is quite evident from the later stages in this specimen that their double nature bears a striking similarity with the split somatic chromosomes. From the very nature of this double thread, more especially as they are strikingly homologous with the somatic prophases, one cannot help coming to the conclusion that the paired threads entering into synaptic process are the sister halves of a single somatic chromosome, and not a lateral pairing of two entirely different somatic chromosomes. From the synaptic contraction a somewhat thick, continuous spireme emerges, which shows also the characteristic double nature. The double univalent spiremes, which are more or less of beaded nature, show their fission very conspicuously when the synaptic knot becomes loosened, and these double spiremes become well distributed in the nuclear cavity, forming what is known as the 'hollow spireme stage'. Gradually, as these 'hollow spiremes' proceed to second contraction stage, they show a tendency to throw themselves into loops. The fission becomes more plainly visible as this process of 'looping over' proceeds, but at no time is it impossible to detect its presence.

Gradually the two branches of the loops become more and more accentuated. These branches of union signify clearly, from their close approximation with each other, the future bivalent chromosomes. After the completion of this process of approximation of two univalents to form bivalents, the spireme is segmented into parts. They show an end-to-end arrangement, but it is difficult at first to say whether the bivalents are open at both ends or at one end only. But at slightly later stages definite cases have been found to be open. We have seen in our material definite cases where each arm of a bivalent chromosome shows distinctly its double nature, which is the result of the approximation of two halves of univalent chromosomes. This is clearly seen in the figures, where some of the *bivalents show distinctly their tetrad arrangement.*

The phenomenon of chromosomic evolution becomes more clear as they come out from second contraction stage and enter into diakinesis. The bivalents are separated into their component univalents as usual. After separation of the daughter chromosomes, they undergo a change which recalls the appearance of longitudinal fission. Subsequent late telophase shows them to be much alveolized, but nevertheless they lose their individuality.

In the foregoing paragraphs attempts have been made to outline briefly the sequence of events in *Doodia*; an attempt will be made later to correlate our observations with those of other workers in this particular line, although with different materials. We are tempted to do this because of the remarkable affinity of chromosomic evolution between these two plants, namely *Osmunda* and *Doodia*. Although in substantial agreement, with the exception of a few minor details, with Miss Digby's (5) conclusion drawn from her observations in *Osmunda*, we have been driven after much hesitation to the definite view that the evidence afforded by this material is far from being in conformity with the several interpretations of Grégoire (12) and Yamanouchi (18). We agree with Grégoire (12), Yamanouchi (18), and Allen (1) that the two halves of the spireme gradually associate in pairs before synapsis, and that the process is completed during synapsis, but we disagree with their interpretation that these two pairs represent two entire somatic chromosomes. Grégoire (12) lays special stress on the point that between the associated filaments at no moment was there produced any sort of fission; according to him, these filaments remain in each pair perfectly distinct one from another, through the whole stage of thick spireme: they are only more or less closely coming together, but they preserve themselves as independent from each other as the two fingers of the clasped hand, one around the other.

This interpretation does not apply in our case, where there is not a single chromosome in which this phenomenon can be observed. On the other hand, we should like to suggest that the graded swellings on the *filaments* become more and more accentuated as they advance in later stages, and certainly there are stages where the paired threads become so much appressed to each other that their individuality seems to be almost lost.

Miss Digby (5) figured and described the thick spireme stage of *Osmunda*, in which one can observe in the filaments 'complete coalescence of the approximated threads'. Her Fig. 59 (*Osmunda*) shows a rather late stage where the chromosomes seem to be quite thick and homogeneous, as the result of complete coalescence. We have not been able in *Doodia* to come across a single case where one can observe this 'complete coalescence of the approximated threads'. As the result of an examination of a long series of serial sections, we are in a position to say that always there may be some traces by which one can distinguish the two separate threads. We have sufficient grounds of evidence from our material to strengthen this consideration. We can hardly, therefore, follow Grégoire (12) in comparing the individual chromosomes to the two fingers of clasped hands, and still less can we, with Miss Digby, admit 'complete coalescence'.

The next objection to Grégoire's (12) and Yamanouchi's (18) interpretation is on the ground that it is rather difficult to follow the series of events given by them, because of the extremely meagre mention of second contraction. We have been gradually forced to the conclusion that the whole interpretation of maturation division depends on full attention being given to this phase. We have endeavoured to show from this material the importance of this stage, as tending to establish our point of view regarding the origin and nature of the bivalent chromosomes.

After a careful consideration of the whole series of events, we have to determine what particular interpretation is best applicable in our material. The nature of the doubleness of the spireme, with its remarkable resemblance to the structures evident in a somatic mitosis, appears to support the interpretation here advocated. That is to say, that as the doubleness found in the early heterotype prophases has a similar appearance and the same origin as found in the somatic prophases, it seems rather inconsistent to hold the view that in the heterotype prophases each parallel side is equivalent to a length of a whole somatic chromosome, and that in the somatic chromosome each side is equivalent only to a length of half a somatic chromosome. If it is true that each side of the split of a somatic chromosome represents only a half length, then it is also true in every respect with regard to the heterotype chromosome.

So the real point is that if the synaptic union is equivalent to the somatic chromosome, what room is there for the second contraction union? If the latter is a fact, Grégoire's interpretation falls to the ground.

On the whole, we find a confirmation of our conclusion—especially with reference to chromosomic evolution of *Doodia*—which is in substantial agreement with the conclusions of Miss Digby (3, 5, 6), Farmer and Moore (8), Farmer and Shove (9), Beer (2), Nothnagel (14), Gates (11). We have observed on our preparations appearances which are very similar to theirs, and almost directly opposed to those of Grégoire (12), Yamanouchi (18), and others.

SUMMARY.

1. Whatever might be the origin of chromatin threads in mitotic division, it is quite evident from the later stages that their double nature bears a striking resemblance to somatic chromosomes.

2. If it is true that each side of the split of a somatic chromosome represents only a half length, then it is also true, in every respect, with regard to the heterotype chromosome.

3. The univalent chromosomes are the result of close approximation of two similar threads, which gradually become concentrated. Throughout the whole evolution they never preserve themselves as independent from each other as the two fingers of the clasped hand, one around the other.

4. The two threads of a univalent chromosome concentrate to a great extent, but nevertheless lose their identity.

5. Bivalent chromosomes are the results of close conjunction of two univalents which, after condensation, become twisted and bend over to form loops.

6. Some of the bivalent segments show *fission and conjunction*.

In conclusion, I take the opportunity of expressing my deep sense of gratitude to Prof. J. B. Farmer, F.R.S., for his invaluable assistance and advice, helpful suggestions and criticisms, and his kindness in affording me facilities in various ways throughout the investigation. I am very grateful, also, to Miss L. Digby, for so kindly placing her exceedingly beautiful preparations at my disposal and also for the helpful interest she has taken. To Prof. R. J. Tabor I am also indebted for many useful suggestions.

THE BOTANICAL LABORATORY,
IMPERIAL COLLEGE OF SCIENCE AND TECHNOLOGY,
LONDON.

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EXPLANATION OF PLATES I–IV.

Illustrating Dr. Sarbadhikari's paper on the Cytology of *Osmundia* and *Doodia*.

All figures were drawn with the aid of an Abbe camera lucida with Leitz 1/12*a* immersion and ocular 18, except Figs. 1–14 inclusive and Figs. 53, 54, and 55, which were drawn with Leitz immersion and ocular 8. Magnification of Figs. 1–14 inclusive and Figs. 53, 54, and 55 × 1088, all others × 2450. Particular care was taken to represent the chromosomes accurately. Figs. 1–14 inclusive are from the somatic divisions of root; the rest are from the sporangiferous apex of fronds.

PLATE I.

Fig. 1. Late telophase. Nucleus, showing the vacuolization of chromosomes. The central portion is seen to be dissolving, leaving the sides as parallel threads with somewhat beaded appearance. There is distinctly a clear space round the nucleus.

Fig. 2. Resting stage. One or more nucleoli may be aggregated in such a way as to present the form of an hour-glass which takes very deep stain.

Fig. 3. A rather early stage of the so-called resting nucleus, where it is provided with delicate network upon which are distributed small chromatin granules.

Fig. 4. Interphase. Note the almost diagrammatic regularity of the parallel aspect of network which is composed of linin reticulum in which are embedded chromatin grains.

Fig. 5. Prophase. After condensation of reticulate unit, a sort of zigzag threads may be formed which,

Fig. 6, soon begin to straighten out, giving rise to definite chromosomes with longitudinal splitting.

Fig. 7. Metaphase. The double nature of chromosomes is remarkably prominent. Those situated in the centre of the equatorial plate are, more or less, a little curved.

Fig. 8. Later metaphase. Note the different degrees of fission.

Figs. 9, 10. 11. Metaphase. Some of the chromosomes are in pairs and appear to cross over each other.

Fig. 12. Anaphase. The separated halves of the daughter chromosomes are on their way to the poles.

Fig. 13. Later anaphase. The chromosomes approximate closely to form a confused and more or less homogeneous mass.

Fig. 14. Very late anaphase in which the chromosomes are fused into masses in the middle of which vacuoles are formed. A cell-plate is formed in the middle of the two nuclei.

Fig. 15. Very early prophase of meiotic mitosis. The chromatin is diffused throughout the linin and staining is comparatively fainter.

PLATE II.

Fig. 16. Slightly later stage. The beaded linin reticulum, which seems more or less of coarse nature, is distributed rather uniformly throughout the nuclear cavity.

Fig. 17. The same in more advanced stage, showing the parallel arrangement of the linin as strands of beads. Some of the granules are seen to be arranged in groups of four.

Fig. 18. The linin is gradually becoming concentrated and appears to withdraw a little from the centre to the framework, leaving a more or less clear space in the centre of the nucleus.

Fig. 19. Further concentrations. The linin strands tend to run parallel to one another.

Fig. 20. In more advanced stages, where the linin fragments assume more definitely their usual spireme character.

Fig. 21. In still more advanced stages, where long lengths may be seen running parallel to one another. This stage is just before first contraction, when the nuclear contents are just beginning to withdraw from the periphery.

Fig. 22. First contraction. The nuclear contents are withdrawing from the periphery and aggregating in the centre irregularly. Fission is almost lost, although traces may be visible here and there.

Fig. 23. Slightly later stage of synapsis; almost all the parts of the nuclear cavity are drawn into the structure of a knot; this is balled up together and entangled at one direction of the nucleus. The association of the threads can be better seen at the free ends.

Fig. 24. The same stage. The spireme is emerging from synapsis in the form of loops. Note the beaded nature.

Fig. 25. The beaded nature of the spireme shows up very clearly. Some of the outer bends of the loops of spiremes are seen to run parallel in parts in such a way that they suggest nothing but close approximation of the two threads of the same filament.

Fig. 26. Almost the same stage as in Fig. 24, but fixed in Flemming's weak solution; the spiremes are seen to be of more or less homogeneous nature.

Fig. 27. The same as in Fig. 26. Note that the less intricate anastomosed threads are thrown into loops.

PLATE III.

Fig. 28. The loosening of the synaptic knot. Note the difference in thickness of the filaments as well as the distinct splittings at intervals in the broad ribbons, more especially at the extremity. The association of threads is now almost complete.

Fig. 29. Early hollow spireme, in which the spiremes, with some of them in loops, are invading the nuclear cavity irregularly.

Fig. 30. Slightly later stage, where every portion of the spireme distinctly shows fission. The fission is not through and through, but closed up at regular intervals.

Fig. 31. A rather advanced phase of hollow spireme, where some of the univalent spiremes, with more pronounced fission, show a tendency to fall over in loops. Some of the filaments, especially those marked *a*, show clearly that the fission goes right along from one end to the other—a striking similarity with the fission of somatic chromosomes (cf. Fig. 7). The sides of the loops, with fission attached to the nucleolus, are seen to run parallel with each other.

Fig. 32. Gradually, in a little advanced stage, these univalent spiremes are seen to run very closely parallel, and the space between them becomes less and less.

Figs. 33, 34. The same in slightly later stage; twisting and looping over and conjunction of univalents are seen; their sides seem to be more thickened and condensed.

Fig. 35. Sometimes just the reverse phenomenon occurs, where the spiremes, after running parallel for a considerable portion in the nuclear cavity, suddenly take a twist and turn in opposite directions.

Fig. 36. Nucleus just before second contraction, where the cavity contains thick spiremes, rather densely aggregated, more in the centre than in the periphery. Note also the gradual obscuring of fission.

Fig. 37. Going into second contraction. The attachment of the filaments to the nucleolus shows the vestiges of fission, and these (namely, the univalents with fission) are proceeding to conjoin with each other by forming loops.

Fig. 38. A nucleus in which second contraction is not quite so accentuated.

PLATE IV.

Fig. 39. Loosening of second contraction.

Fig. 40. Some of the bivalent segments have come out of second contraction and show *fission and conjunction.*

Fig. 41. A section of nucleus coming out of second contraction, showing bivalent segments. They are not yet fully individualized.

Fig. 42. Slightly later stage in which the bivalent segments are more definite.

(NB.—Figs. 39, 40, 41, and 42 are all from the same sporangium.)

Fig. 43. Shows a nucleus where the bivalent segments are mostly individualized and begin to show some of the typical heterotype shapes.

Fig. 44. Showing still more definite bivalent chromosomes. Note the different degrees of combination into bivalents.

Figs. 45, 46. Almost the same stage as in Fig. 44. Many of the bivalent chromosomes demonstrating fission in their univalents, somewhat concentrated. Note the different forms assumed by the bivalents. In chromosome A of Fig. 46 note also the vestiges of fission of univalents of each bivalent combination.

Fig. 47. Slightly later stage in which the fission is less visible.

Fig. 48. Early diakinesis, showing the commencement of spindle or striation of cytoplasm. Note the bivalent pairs, which are such familiar figures in diakinesis. Most of them are in the form of an X.

Fig. 48. Late diakinesis; fully developed, somewhat concentrated chromosomes. Some, showing fission, are scattered towards the nuclear periphery.

PLATE V.

Fig. 50. Still more concentrated chromosomes, in which fission is almost obliterated, preparing to go on to the spindle. The bivalent chromosomes have concentrated and condensed to such an extent that they look like monstrous massive patches.

Fig. 51. The equatorial plate stage of the first meiotic division.

Fig. 52. Anaphase. The chromosomes are at first condensed and crowded in such a way that they look like so many deeply stained groups of patches.

Fig. 53. Gradually from the condensed mass fragments come out which soon become alveolized.

Figs. 54, 55. The longitudinal splitting of each daughter chromosome becomes more and more separated, and finally gives rise to the vacuolated appearance which is so familiar in somatic chromosomes.

Fig. 56. Telophase of second meiotic mitoses, showing three daughter nuclei. The structure of the chromosomes which is lost by beading and alveolization presents a remarkable affinity to heterotype and somatic telophase.

Fig. 57 shows three adjacent sporangia where three widely divergent stages are visible. Sporangium A shows most of the nuclei are in synapsis, sporangium B shows them to be in second contraction stage, while in sporangium C we get late telophase of heterotype division.

ERRATA

Annals of Botany, Vol. XXXVIII, No. CXLIX,
January 1924.

Page 24, line 15 *for* ‘Ricines d'allium’ *read* ‘Racines d'Allium’.

Page 24, add to references:—

13 A. Martens, P. (1922): Le Cycle du Chromosome Somatique dans les Phanérogames. La Cellule, XXXII, pp. 333-413.

On the Life-history of Harveyella pachyderma and H. mirabilis.

BY

H. H. STURCH.

With twenty-two Figures in the Text.

THE genus *Harveyella* includes at the present time two species, both common on the southern coasts of England and Ireland. One of these, *H. mirabilis*, parasitic on *Rhodomela subfusca*, was described in this Journal, March 1899; the other, *H. pachyderma*, parasitic on *Gracilaria confervoides*, is the subject of the present paper, which has been delayed by my having been stationed out of England for twenty-one years.

Harveyella pachyderma was found parasitic on *Gracilaria confervoides* at Stoke's Bay, Gosport, in 1896, and identified by Mr. E. M. Holmes as *Choreocolax pachyderma* (Reinsch) (1). Cystocarpic specimens proved it to be a *Harveyella*, and it was placed in that genus as *H. pachyderma* (Holmes and Batters). In the paper on *H. mirabilis* (4) the genus was removed from the Gelidiaceae, in which it had been placed by Schmitz (2), and transferred to the Gigartinales, while Oltmanns in 1905 (3) placed it at the beginning of that group. The more recent examination of the two species found near Cork Harbour and at Plymouth has confirmed the results of my previous paper (4), and shown that the apparently closely-allied species *H. pachyderma* differs considerably in the development of the cystocarp. *H. mirabilis* and *H. pachyderma* are very similar in appearance when fresh, appearing as small white cushions, hemispherical or slightly flattened, and attached to their hosts by a very short thick stalk. Each plant, external to the host, is, when fully grown, about the same size as the mature cystocarp of *Gracilaria confervoides* (Fig. 1).

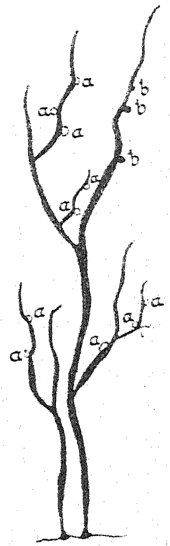

Fig. 1. *Gracilaria* with *H. pachyderma*. *a*, *H. pachyderma*; *b*, cystocarp of *Gracilaria*. Natural size.

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Both plants are true parasites without colour of their own, and both often appear coloured by the many minute filamentous Florideae which grow in and on the thick gelatinous coat enclosing the *Harveyella*. The differences in the vegetative structure of the two species are so slight that I shall deal with them both together, then fully with the development of the cystocarp of *H. pachyderma*, and finally point out the differences in the development of *H. mirabilis*.

Each plant consists of two parts—

1. Branched filaments growing among the cells of the host, and absorbing food material from this host.

    (*a*) In *H. mirabilis* these parasitic filaments are copiously branched into (in section) fan-shaped masses, sometimes as large as the whole external cushion. These filaments frequently make their way into the interior of the cells of the host.

    (*b*) In *H. pachyderma* the parasitic filaments are long, less branched, and follow only the middle lamella region between the cells of the host. They are joined by secondary connexions with the contents of the inner peripheral cells of the *Gracilaria*, and occasionally with those of the central cells also.

2. These parasitic filaments give rise, outside the host plant, to the external cushions, each of which, when mature, consists of a small-celled peripheral layer four to five cells deep, passing over into the central mass of branched filaments of larger cells. The average diameters of the outer cells of the peripheral layer are from 4μ to 6μ, increasing in the inner layer to 10μ to 20μ. The cells of the central filaments vary in diameter from 30μ to 70μ.

    (*a*) In *H. mirabilis* these central cells are more regularly arranged and the intercellular matter is not relatively thick.

    (*b*) In *H. pachyderma* this intercellular matter is much thicker, and the filaments are more irregular.

The outer gelatinous coat surrounding the external cushion in *H. mirabilis* is comparatively thin and shows no lamination when stained, but in *H. pachyderma* it is as thick as the whole peripheral layer, and with methylene blue shows a distinctly laminate structure in its outer third; the inner portion, although continuous with the intercellular matter of the central part of the cushion, does not resemble it in staining readily with central lamella stains.

LIFE-HISTORY OF *H. PACHYDERMA*.

It is abundant from November to March on *Gracilaria confervoides* growing immediately below low-water mark, but disappears from June to October, although the host plant is still abundant in the same region.

Plants bearing antheridia, procarps, or cystocarps occur from November to February, tetrasporic specimens from January to March. Less than 1 per cent. from November to the middle of January bear tetraspores, while about 10 per cent. of the plants occurring in March bear sexual organs. Undoubtedly the carpospores set free in late November and December give rise to the tetrasporic plants of the later winter months. Crowds of tetraspores are set free in February, but no young plants appear before, at the earliest, October. I have collected and sectioned a large number of *Gracilaria* growing in the summer at places where *Harveyella* is common in the winter months, but found no sign of the parasite developing within its host. A few stray specimens found on the beach after heavy storms led me to suspect that the summer increase of temperature compelled *Harveyella* to grow in deeper water. Recently, by the kind permission of Dr. E. J. Allen, F.R.S., I have been able to examine the dredgings at the Plymouth Marine Biological Station, and have found a small but constant number of *H. pachyderma* on *Gracilaria confervoides* dredged from depths of eight fathoms or more, throughout the whole year. The results of those cast up on the beach after storms in the summer, and of my own attempts at dredging near Cork, together with the results at Plymouth, give the following monthly averages from deeper water only:

	Antheridial.	*Cystocarpic.*	*Tetrasporic.*
January	7	15	3
February	0	1	10
March and April	2	1	25
May and June	6	8	0
July	3	9	1
August	1	1	8
September	0	2	9
October	5	12	0
November	6	13	0
December	4	16	0

These numbers agree with the theory that *H. pachyderma* develops in the deeper water during the whole year at much the same speed as in the surface water in the winter months, and that its annual life-history is as follows:

November to January. Numerous antheridial and cystocarpic plants in shallow water, and a smaller number of the same in deeper water.

February to April. Equally numerous tetrasporic plants in shallow water and a smaller number in deeper water.

May to July. Antheridial and cystocarpic plants in deeper water only.

August to September. Tetrasporic plants in deeper water only.

October. The tetrasporic plants die out during October, giving rise to the antheridial and cystocarpic plants in both shallow and deeper water.

In the above table the term cystocarpic includes also young plants bearing procarps.

Although *Gracilaria* is a smooth plant of firm texture, and not copiously branched, crevices are left in the surface by the emergence of carpospores or tetraspores, which would easily allow the entrance of the spores of *Harveyella*. When *Gracilaria* plants infected with *H. pachyderma* are found in the small pools left at low water, I have seen as many as three hundred, all tetrasporic, on a single host plant whose total branches measured less than forty inches, and sectioning showed that one hundred and eight at least had each arisen from a separate spore. These must have been the result of the setting free of a cloud of carpospores at low water in this small pool. Set free at higher water when the pool was only part of the moving tide the spores would be scattered, and would develop, as is most frequently the case, into one or two *H. pachyderma* on each *Gracilaria*.

The earliest stage of *H. pachyderma* yet found is shown in Fig. 2. This is one of a series of sections which included the whole parasite, and is the most central of them. The spore from which it arose has evidently penetrated some two or three cells deep into the host before developing, but the aperture by which it entered could not be traced.

The parasitic filaments grow rapidly along the middle lamella in the host's cell-walls, and soon the growing cells push through the periphery to form a minute white cushion on the exterior (Fig. 3).

All the filaments, internal and external, grow in the same way, the distal cell of each filament dividing by a more or less transverse wall, the lower half increasing in size, the upper elongating and dividing again. Or the distal cell may divide by a curved oblique wall, producing a sub-dichotomous branching.

The thick gelatinous outer coat of *H. pachyderma* is rather impervious to liquids; a *Gracilaria* exposed to the sun until withered still bears the cushions of *Harveyella* plump and moist. If a frond of *Gracilaria* bearing a *Harveyella* be plunged into a suitable watery or alcoholic stain, and sectioned, the *Gracilaria* cells and cell-walls will be most stained; and the *Harveyella* will have absorbed the stain through the host, its own peripheral region staining last. Attempts to impregnate *Harveyella* with paraffin, however carefully carried out, nearly always result in sections which are unrecognizable as *Harveyella*. The outer portion of this gelatinous coat of the *Harveyella* is continuous with, and has similar staining reactions to, the outer coat of the host plant. As this latter is not ruptured in any way by the emergence and subsequent growth of the *Harveyella*, apparently its substance is continually added to by the parasite. While still very young the antheridial, cystocarpic, and tetrasporic plants are indistinguishable, but as soon as a distinct peripheral layer becomes visible the development of antheridia, procarps, or tetrasporangia commences.

The procarp is cut off from a distal peripheral cell in the ordinary way, the new cell elongating at its apex (Fig. 4, *a*). Any peripheral filament may give rise to procarps, which are soon developed along the entire surface of the external cushion. The cell beneath the procarp, from which the latter was cut off, continues its functions as a peripheral cell, while the procarp grows downward at its base on the side and cuts off one small cell; no more cells appear, and the carpogonial branch in *H. pachyderma* is thus two-celled. During the examination of a very large number of procarp-bearing specimens of this plant (and the average number of healthy procarps on one plant is about 2,100), I have seen two four-celled and five three-celled carpogonial branches: thus it is possible that before reduction due to its parasitic habit the carpogonial branch may, as in *H. mirabilis*, have also been normally four-celled.

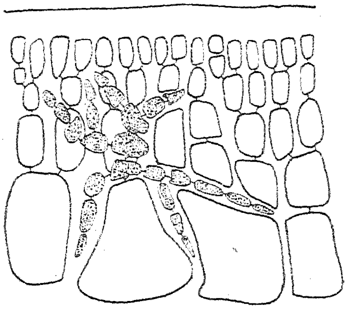


FIG. 2. Young *H. pachyderma* before emergence from host. Zeiss 4 mm., oc. 8. *Harveyella* cells are shaded.

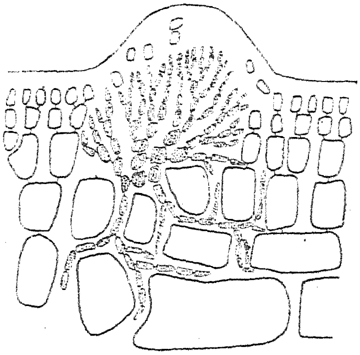


FIG. 3. *H. pachyderma*, somewhat older. *Harveyella* cells shaded. Zeiss 4 mm., oc. 4.

As the external cushion grows and the procarps are buried more deeply in the periphery, other procarps are developed on the younger peripheral cells until there are several on the same filament (Fig. 4).

The older unfertilized procarps usually degenerate and disappear, the process beginning at the trichogyne, but occasionally the procarp itsel persists after the disappearance of its trichogyne, and apparently resume its place as an ordinary thallus cell. The great majority of the procarp produced reach the surface of the plant and project into the wate (Fig. 4); a few fail to penetrate the outer part of the external coat, thes bending at right angles and still growing in length for a short distance and some twisted or even branched specimens are occasionally found The part of the trichogyne projecting into the water is slightly swollen and surrounded by mucilaginous matter. I have not seen an undoubte case of the attachment of a spermatium, possibly owing to the delicac of the projecting trichogyne ends, which are easily broken off in sectioning, but in *H. mirabilis*, where these organs are stouter, I have frequently seen them with spermatia attached. The average dimensions of the mature procarp with its accessory trichogyne in *H. pachyderma* are as follows:

Total length, 110 μ.
Breadth near surface of plant, 5 μ.
Length of projection into water, 12 μ.

Before any development of the carposporophyte takes place there is

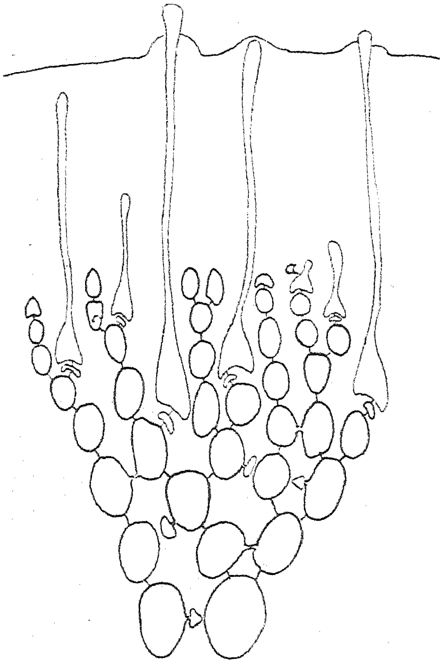


FIG. 4. *H. pachyderma.* Stages in the development of the procarp. 4 mm., oc. 4.

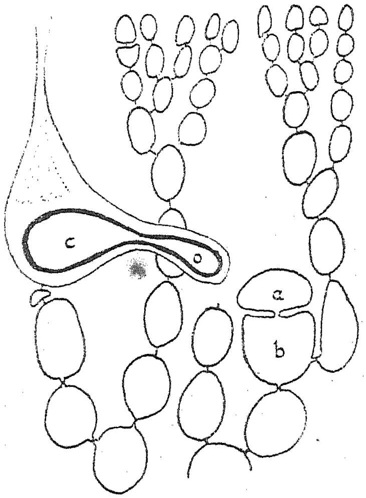


FIG. 5. *H. pachyderma.* 4 mm., oc. 8. *a.*, auxiliary cell; *b.*, basal cell; *c.*, carpogonium; *o.*, ooblastema.

ı considerable formation of secondary pit connexions in the inner layer of ɔeripheral cells, and in those of the central thallus immediately beneath. A similar process takes place in many other Florideae, and the behaviour of the nuclei and the formation of the connexions does not in this case liffer from the method described by Oltmanns (3). As has been pointed ut by Church (5), these connexions are made possible by the softness of he matter separating the cells; and they enable the food material to be apidly concentrated where required for the production of masses of spores. A similar process takes place in *H. pachyderma* before the formation of etrasporangia, and to a smaller extent before the development of the ntheridia.

Owing to the development of these secondary pit connexions, and the movements of the daughter nuclei in connexion with them, all the cells of the central part of the thallus become coenocytic, as also do some of the older cells of the parasitic filaments.

The majority of the procarps develop no further, but one or more of them become fertilized (as far as can be ascertained when the actual passage of the male nucleus has not been seen). The zygote then sends out a short primary ooblastema, sometimes two-celled (Fig. 5). At about the same time, the auxiliary mother-cell, which is one of the lower cells of a very near, if not the nearest, peripheral chain, divides by a transverse wall into a lower basal cell (*b*) and an upper auxiliary cell (*a*) (Figs. 5, 6). Occasionally this auxiliary cell pushes out a small projection towards the approaching ooblastema; fusion takes place (Fig. 6), and the fusion cell is then at first developed much as in the typical Ceramiales.

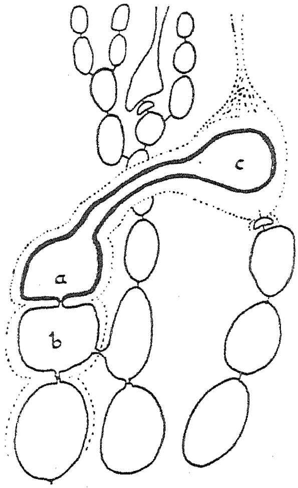


FIG. 6. Fusion of ooblastema with auxiliary cell. *a.*, auxiliary cell; *b.*, basal cell; *c.*, carpogonium.

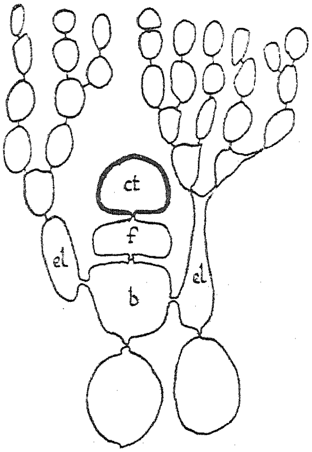


FIG. 7. Division of auxiliary cell after fusion into foot cell, *f*, and central cell, *ct*. *b*, basal cell; *el.*, elongating cells.

The fusion cell is soon divided transversely into a lower foot cell and an upper central cell, the latter containing a nucleus descended from the zygote. This rapidly divides (Fig. 8), giving rise to the secondary ooblastema. At the same time the cells at the base of the periphery commence to elongate, and in all cases this process begins directly after the fusion of the primary ooblastema with the auxiliary cell, the first cells to lengthen being connected, primarily or secondarily, with the basal cell (Figs. 7 and 8, *el.*). The secondary ooblastema grows rapidly at about the same level as the bases of these lengthening cells, and the elongation of these basal peripheral cells accompanies its growth around the whole thallus, commencing in each cell just before it is reached by the advancing distal end of the ooblastema (Fig. 9).

A gametophyte cell will often send out three or four processes which become connected not only with the advancing sporophyte, but also with the bases of the elongating cells, and these processes enlarge until finally a layer is produced consisting of cells of the secondary ooblastema and the gametophyte fused together to a trophocyte from which the growing gonimoblast clusters spring.

In Fig. 9 the cell *a*, which is putting out a process to meet the distal cell of the advancing ooblastema, will itself develop into an elongated cell, and the similarly situated cell *b*, which is not putting out any process, will also become an elongated cell. The secondary connexions do not follôw any rule.

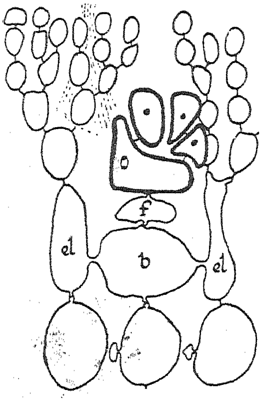


FIG. 8. Early division of central cell. *b.*, basal cell; *el.*, elongating cell; *f.*, foot cell.

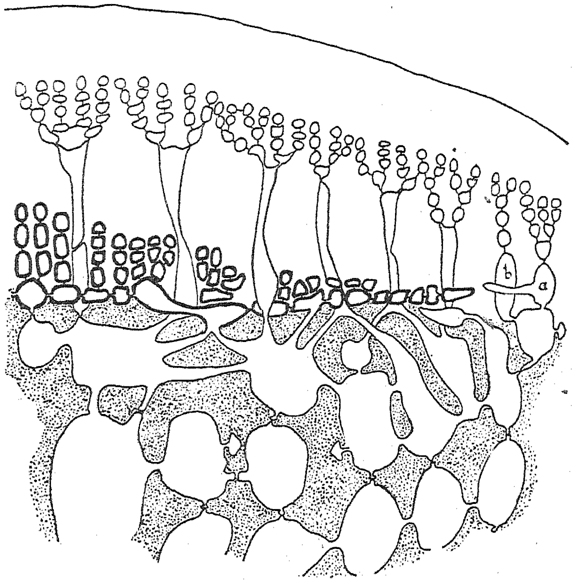


FIG. 9. *H. pachyderma.* 4 mm., oc. 4, reduced. Part of young carposporophyte; secondary ooblastema (thick outlines) with secondary connexions to much-fused cells of gametophyte. The dotted portions are gelatinous cell-walls.

A few cells behind the advancing distal end the cells of the horizontal ooblastema begin to develop gonimoblast clusters of carposporangia, branching in all directions. In *H. pachyderma* the branching is almost confined to the base of the cluster, thus producing nearly vertical rows of carposporangia. Each cluster is separated from the neighbouring clusters by a moderately thick gelatinous mass, and each carposporangium is surrounded by a thin wall of the same material.

Eventually the carposporangia, each containing one uninucleate carpopore, encircle the whole thallus. The surrounding gelatinous material, now for the first time, becomes granular in appearance when stained with safranin methylene blue.

The diameters of the carpospores of *H. pachyderma* vary within the wing limits, 9 by 7 μ, 10 by 15 μ.

e cells of the raised periphery also show changes, becoming much ened and joined by secondary connexions. The gelatinous substance

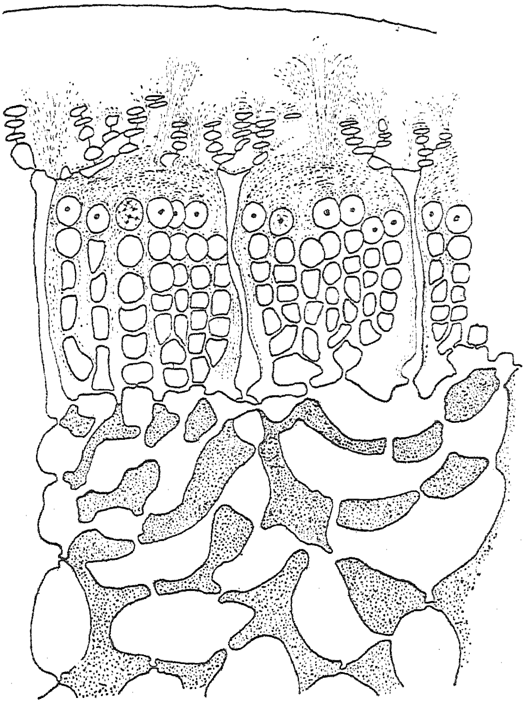
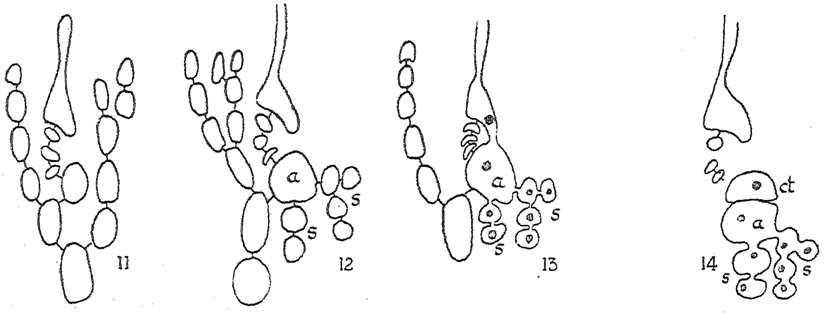


FIG. 10. *H. pachyderma.* Part of mature cystocarp. The dotted portions represent readily stained intercellular matter and disintegrating polysaccharide material. The secondary ooblastema has fused with the gametophytic cells beneath it to a trophocyte extending all round the thallus.

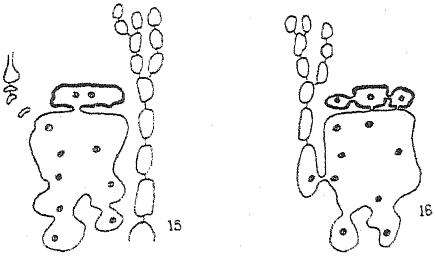
in which they are embedded becomes, when the cystocarp is mature, visibly granular on staining, the disintegration commencing as lines arising between the tufts of peripheral cells and especially round the remains of old procarps (Fig. 10). This disintegration always proceeds from within outwards, and arely reaches the surface. The intercellular material increases considerbly, and stains rapidly and very deeply with middle lamella stains, but nally the mature carpospores lie in a space which shows no coloration ith either middle lamella or mucilage stains, all signs of the stainable atter formerly surrounding them having disappeared. The still tough d, by the growth of the cystocarp, much-strained outer coat breaks away, d the carpospores are all set free into the water together.

Harveyella mirabilis differs from *H. pachyderma* in the carpogonial branch, and in the details of the nutrition of the ooblastema.

In *H. mirabilis* the carpogonial branch is without exception four-celled. The peripheral cell supporting the carpogonial branch acts as the auxiliary cell, and there is no further division into basal and auxiliary cells. But this cell, before fertilization of the carpogonial nucleus, cuts off from its lower part two small cells which develop into short sterile branches, one of two, the other of four cells (Figs. 12 and 13, *s*.). After fertilization, fusion takes place by means of a very short ooblastema, and a central cell is cut off (Figs. 13, 14), while the lower part—the foot cell—and the cells of the sterile branches widen their connexions and eventually become fused into a very large coenocytic cell (Figs. 15, 16, 17), often joined by broad secondary connexions to the nearest cells of the gametophyte.



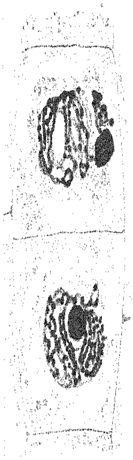
FIGS. 11–14. Stages in the development of the procarp and the auxiliary cell of *H. mirabilis*. *a*., auxiliary cell ; *ct*., central cell ; *s*., sterile filaments. 4 mm., oc. 4.



FIGS. 15 and 16. *H. mirabilis*. Development of auxiliary and central cells. Sporophyte with thicker outlines. 4 mm., oc. 4.

The central cell, containing the descendant of the zygote nucleus, quickly divides, producing, as in *H. pachyderma*, secondary ooblastema, which in this case have no further connexion of any kind with the cells of the gametophyte (Fig. 17); they derive all their nourishment from the coenocytic food reservoir formed from the two small sterile branches and the lower part of the auxiliary cell.

In this plant in the gametophyte there are none of the secondary



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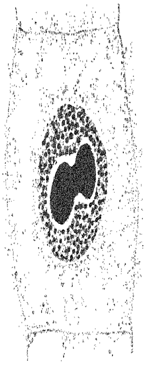

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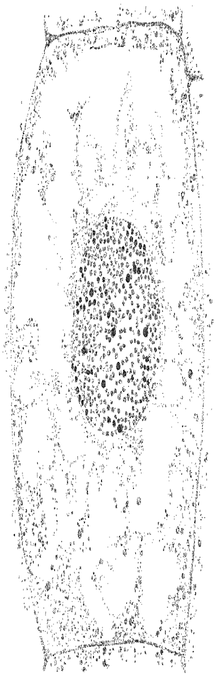

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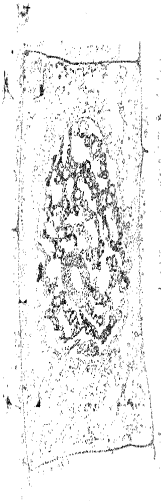

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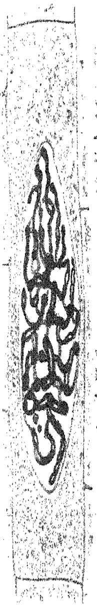

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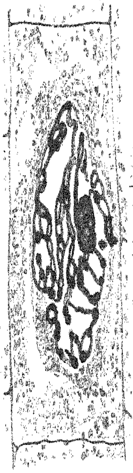

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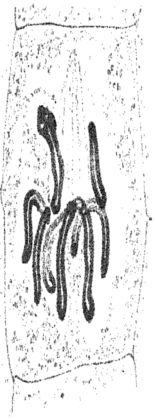

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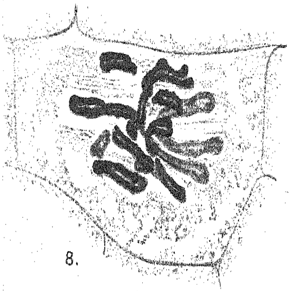

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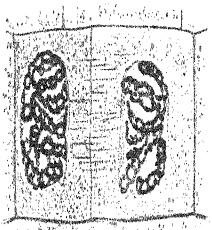

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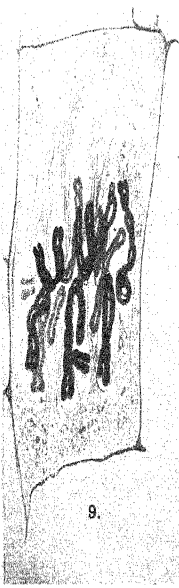

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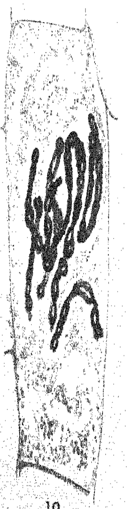

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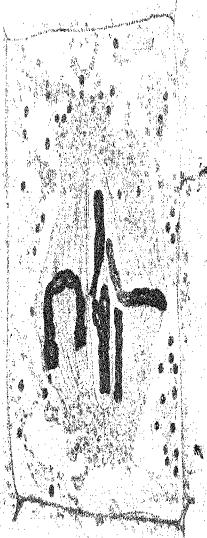

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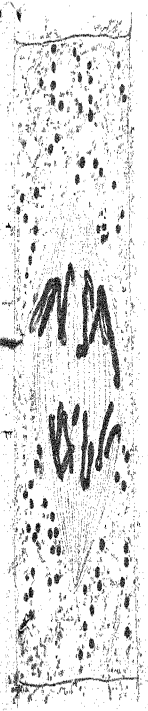

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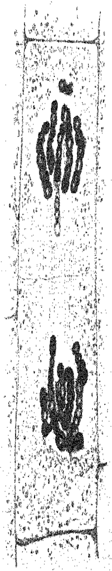

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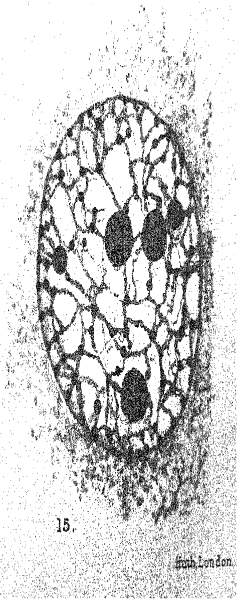

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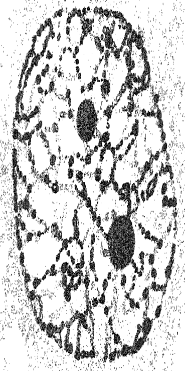

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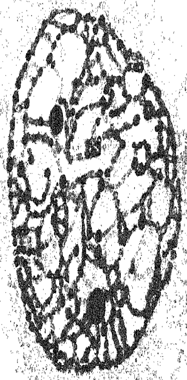

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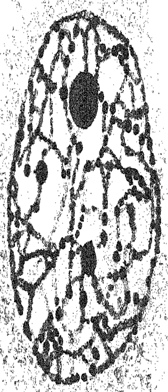

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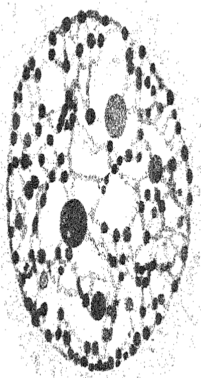

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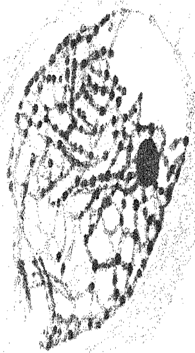

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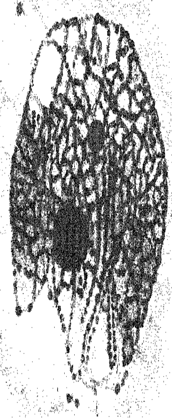

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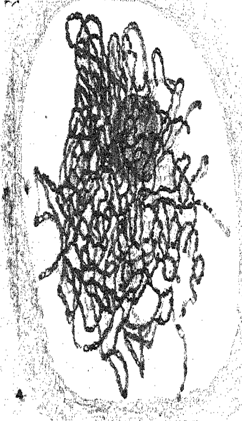

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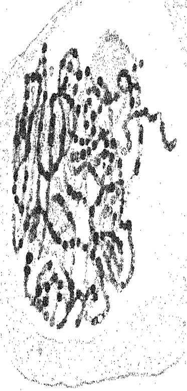

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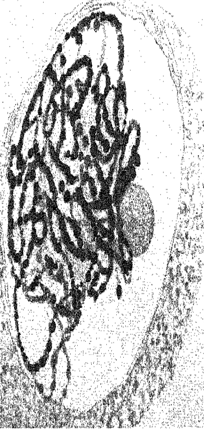

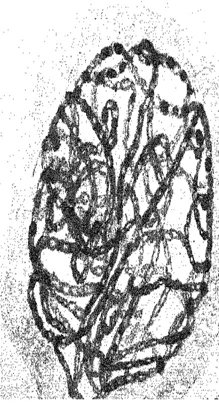

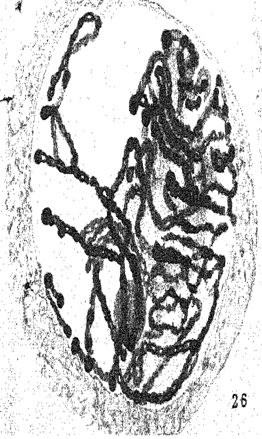

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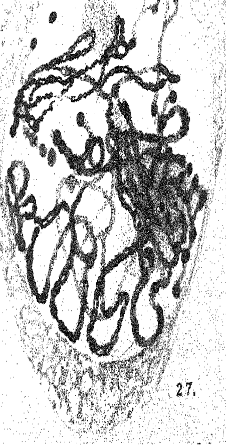

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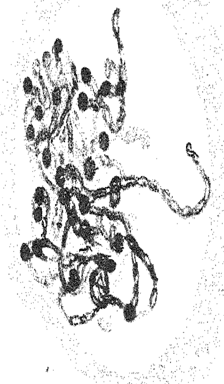

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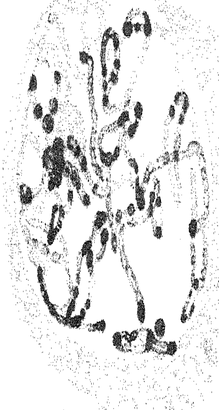

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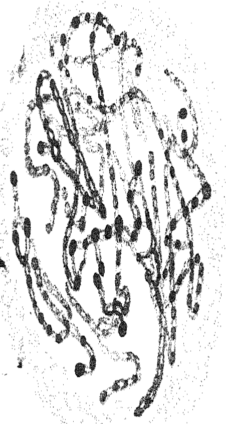

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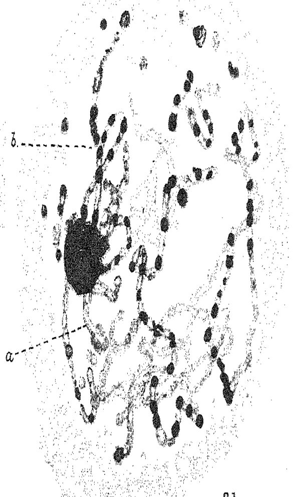

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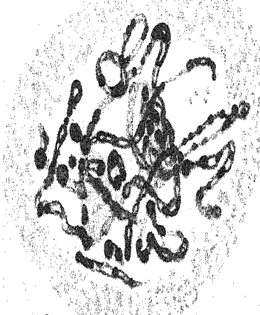

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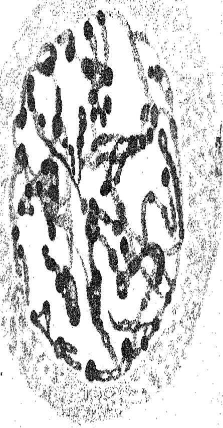

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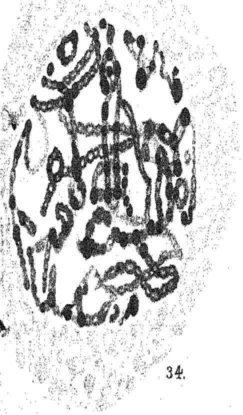

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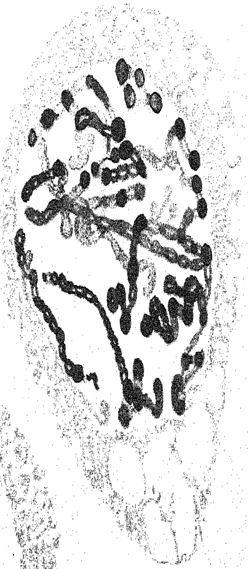

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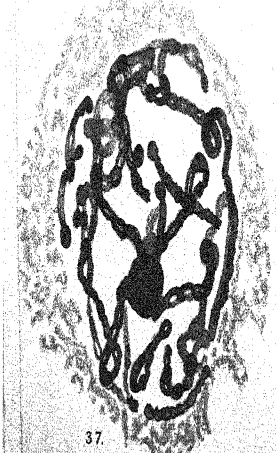

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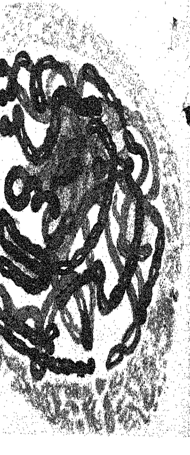

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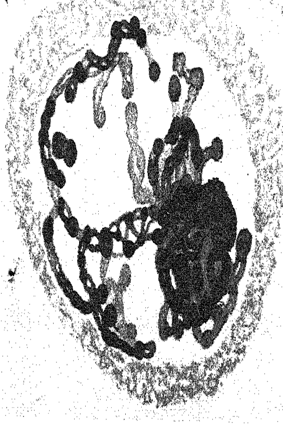

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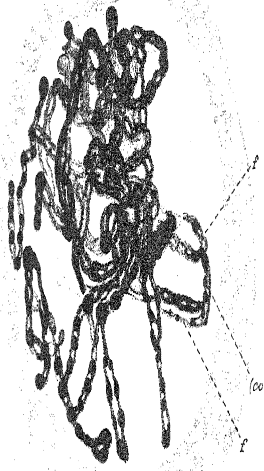

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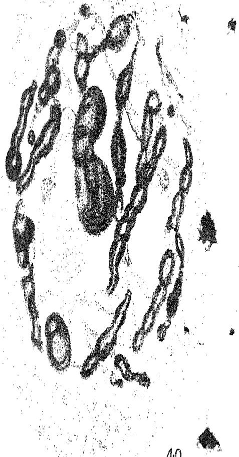

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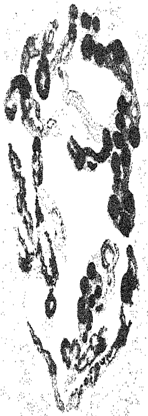

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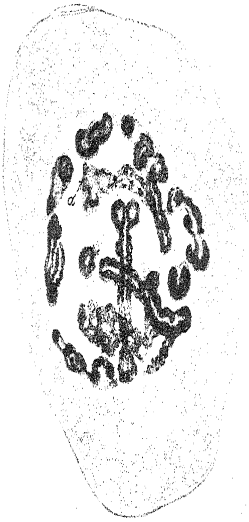

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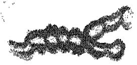

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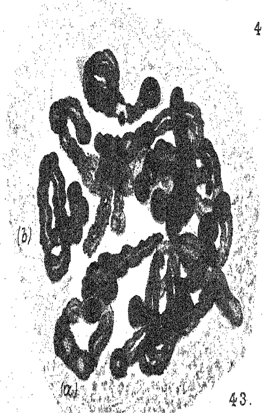

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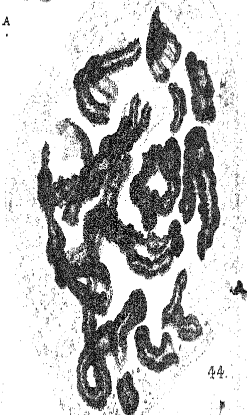

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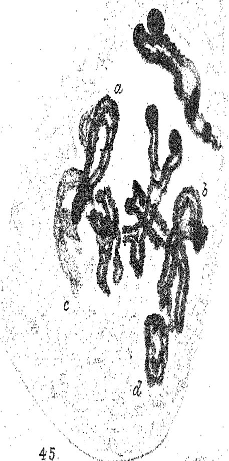

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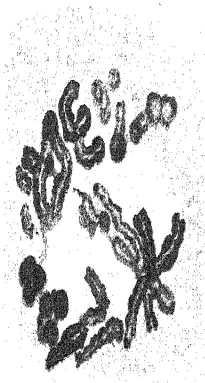

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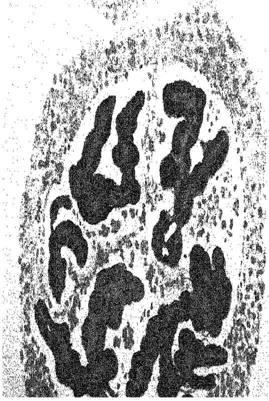

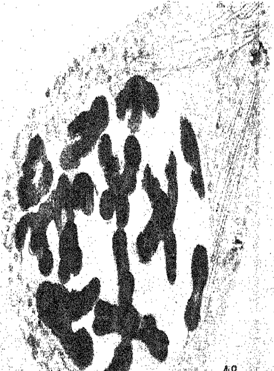

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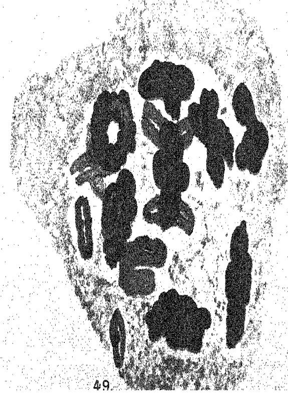

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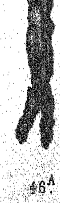


46A.

connexions which are so common in *H. pachyderma*, and all the cells of the thallus remain uninucleate.

The gonimoblast clusters of carposporangia are developed similarly to those of *H. pachyderma*, but the branching is from a narrower base, and is continued farther up the branches, thus producing clusters whose branches radiate outwards (Fig. 17).

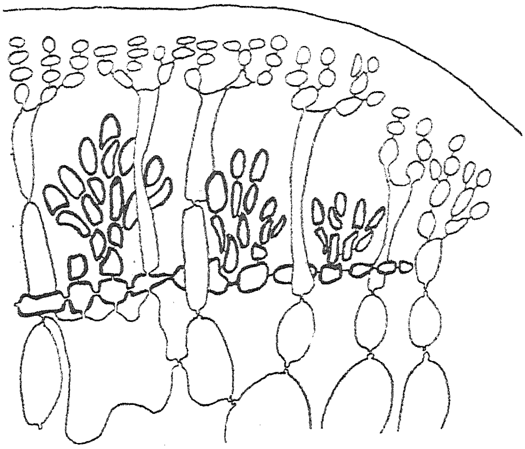


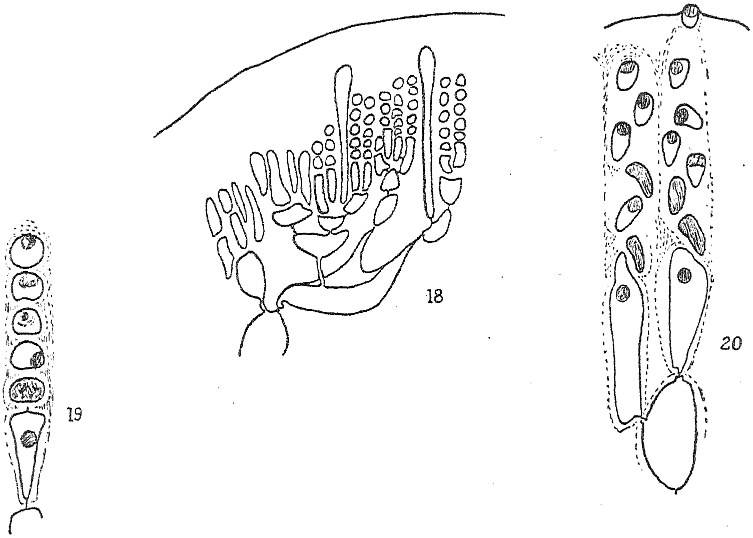
FIG. 17. *H. mirabilis.* Small portion of young cystocarp. Sporophyte with thicker outlines. 4 mm., oc. 4.

The carposporangia of *H. mirabilis* are more oval than those of *H. pachyderma*, their average diameters being 25 by 10 μ, 20 by 7 μ.

ANTHERIDIA.

The antheridia in both plants are produced by the rapid division of the distal cells of the young periphery (Fig. 18).

Small tufts of more or less elongated cells are first produced, which then give rise to the spermatia. The cells of these tufts are usually as shown in Fig. 18, but in both plants a variation appears, the antheridia being produced as spreading tufts of narrow cells radiating from a narrow base. The nucleus of each narrow cell divides, one daughter nucleus moving to the upper end, the other to the lower. The upper proceeds outward into the gelatinous material of the outer coat of the thallus, and the lower repeats the process from six to eight times. In Fig. 20 a spermatium has passed through the outer coat and is just escaping into the water; this occurs in both plants occasionally. Usually the outer coat becomes so weakened by the mass of spermatia formed all round the thallus that it gives way at some point—flies off like a broken spring—and all the spermatia are set free together. In *H. pachyderma* the spermatia are cut off regularly, by transverse walls, one after the other (Fig. 19), but in *H. mirabilis* they are produced obliquely and alternately (Fig. 20).



FIGS. 18–20. 18. *H. pachyderma.* Antheridia and spermatia. 4 mm., oc. 8, reduced. 19. *H. pachyderma.* Antheridia and spermatia. 2 mm., oc. 8. 20. *H. mirabilis.* Antheridia and spermatia. 2 mm., oc. 8.

TETRASPORANGIA.

These are developed in the same way in both plants.

A distal cell of a very young peripheral chain divides as usual by an oblique wall (Fig. 21); the narrow cell *a* is then usually cut off from its basal part, elongates, and may divide once more. The oval cell *b* is the tetrasporangium and rapidly enlarges; it divides cruciately, producing four uninucleate tetraspores, the transverse division appearing first. After the four spores are thus produced, still contained in the wall of the tetrasporangium, a narrow line just outside this wall commences to stain deeply with aniline blue or gentian violet, while at the apex appears a cap of similarly stained material, and immediately above this radiating lines. The gelatinous substance around is softened, especially near the apex, and the tetrasporangium moves through the outer coat until it reaches the water. The track through which the tetrasporangium has passed stains with aniline blue, and an old tetrasporic plant shows a large number of such tracks. Often the tetraspores escape from the tetrasporangium wall before it reaches the water; they then flow out one by one (Fig. 21, *c*). Although the substance of this outer gelatinous coat is altered when either tetrasporangia, carposporangia, or antheridia are developed within it, the changes are not the same. Acted on by the tetrasporangia the product stains with aniline blue, but this stain has no effect when carpospores or antheridia are developed. Plants bearing apparently both antheridia and procarps are occasionally found, but always one half of the thallus bears procarps only and the other half antheridia only.

These specimens are the product of separate spores, which have chanced to develop very near to each other. The two external cushions, emerging on the surface of the host, in close proximity and at the same time, will naturally have a few, and only a few, of the host's peripheral cells between them.

As the two cushions grow, their outer coats coalesce and when mature appear as if they enclosed one plant, half male and half female. But invariably between the two halves the outer coat is slightly dented inwards, and directly under this dent is the little bunch of *Gracilaria* peripheral cells which originally divided the two young cushions (Fig. 22). Cases of two male or two female plants growing together are common, and always show this dent and the peripheral cells beneath it. Tetrasporic plants are not found with either cystocarpic or antheridial plants in a similar combination, as they so seldom develop at the same time of the year. Thus in *Harveyella*, unlike many other Florideae, the cystocarps, antheridia, and tetraspores are invariably developed on separate individuals.

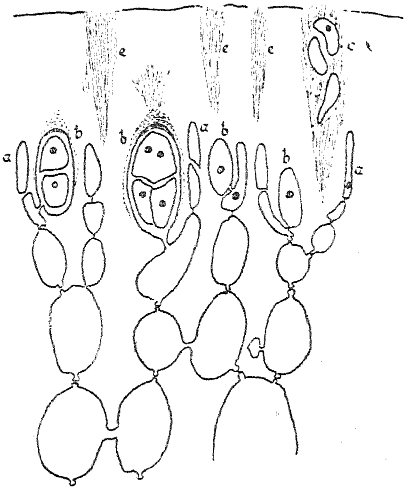


FIG. 21. *H. pachyderma.* Tetrasporangia. 4 mm., oc. 8. *a*, sterile cells; *b*, stages in tetrasporangia; *c*, tetraspores escaping; *e*, tracks left after tetraspores have escaped.

After the escape into the water of tetraspores, carpospores, or spermatia, *Harveyella* dies. It is not at first clear why this should happen; the store of available food in the host plant, relatively to the size of the parasite, is very large, the parasitic filaments are apparently healthy, and it seems possible for them to give rise to a new external cushion. This, however, does not occur. From the first appearance of the *Harveyella* outside its host there are signs of an excess in the amount of carbohydrate conducted from the parasitic cells to the external cushion, and by the time the carpospores are developing not only is the intercellular matter very bulky, but every cell of the central tissue is full of amylodes. When the carpospores, &c., are mature, the contents of these central cells have much decreased, the intercellular matter has grown till it exceeds the total bulk of the cells themselves, and the numerous pit connexions seem to cease to be available.

The parasitic filaments have hitherto been comparatively thin walled and their contents not granular, but they also now become filled with amylodes; the ends of the filaments often branch profusely and become surrounded by increasing masses of waste polysaccharides; the external cushion is unable to use the carbohydrate which the parasitic cells are still accumulating. *Harveyella* is, from a reproductive point of view, very efficient: every plant produces carpospores, tetraspores, or spermatia over the whole surface of the external cushion; hence when these are set free there is no periphery left. In the cystocarpic plant all the peripheral cells and outer coat, raised on the elongated cells, break away before the carpospores escape; in the antheridial plant nearly all the peripheral cells have been developed into antheridia, and the outer coat breaks away in one piece to set free the spermatia; in the case of the tetraspores practically all the peripheral cells have developed into tetrasporangia, and the outer coat is gradually destroyed by their passage through it. In each case the exhausted central cells and the softened intercellular matter are exposed to the sea, and rapidly disappear under the action of bacteria and other organisms.

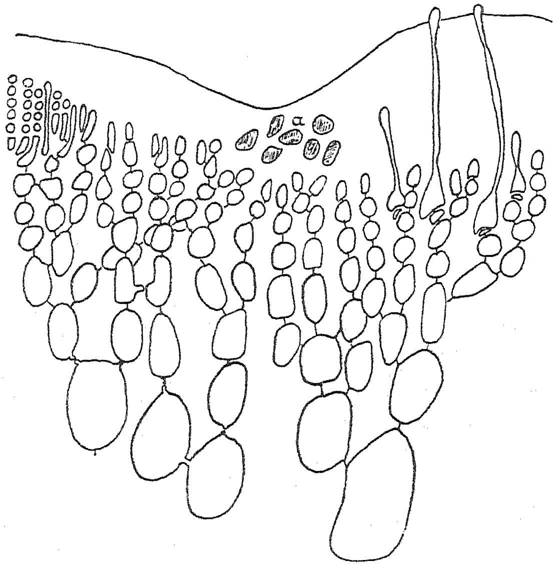


FIG. 22. *H. pachyderma.* Junction of separate male and female plants. *a*, small group of *Gracilaria* peripheral cells. 4 mm., oc. 4, reduced.

The parasitic filaments still remain in the host plant. In some the *Gracilaria* cells near, naturally empty, and exposed to the sea, collapse and the whole frond breaks off. Very frequently this is not the case; the host remains healthy, and the parasitic cells, full of amylodes, have their further development stopped by enclosure in a relatively thick mass of laminated carbohydrate material, much resembling the original external layers of the outer coat of the *Harveyella* cushion. Filaments in all stages are seen enclosed, from two to fifteen cells in length, with cells shrivelled and dead or still apparently alive. The enclosing layers are often three times as broad as the diameter of the filaments, and are probably the product of the parasitic cells themselves. Other species figured by Reinsch in 'Contributiones ad Algologiam et Fungologiam' are named as follows:

1.	*Choreocolax*	*Rabenhorstii*,	parasitic on	Delesseriae.
2. . .	„	*Americanus*,	„	*Lophura Royana subfusca.*
3.	„	*Macronemia*,	„	*Laurencia pinnatifida.*
4.	„	*tumidus*,	„	*Ceramium involutum.*
5.	„	*destructor*,	„	*Gigartina Teedii.*

None of these has been investigated: any of them may be other species of *Harveyella.* I have not been able to find any of these plants, which are stated to be common on the coasts of North America.

One other plant first described by Reinsch in the same volume, and of which I have only seen one very young specimen, is *Choreocolax Polysiphoniae,* parasitic on *Polysiphonia fastigiata*, described by H. M. Richards (6). This paper was incomplete, but it definitely proved that the parasite was not a *Harveyella*, though closely resembling it in structure and appearance. The cystocarpic cushions are lobed, and each lobe contains one cystocarp, consisting of a conceptacle with an opening to the sea. This conceptacle is bounded by a layer of flat tabular cells, and from these arise protuberances on each of which grows a single carpospore and a sterile filament. The drawings in this paper remind me of a *Galaxaura*-like plant, much reduced by a parasitic habit and enclosed in a gelatinous envelope.

SUMMARY.

1. *H. mirabilis* and *H. pachyderma* are true algal parasites, belonging to the Florideae, and growing respectively only on *Rhodomela subfusca* and *Gracilaria confervoides.*

2. In habit, external morphology, and somatic details as far as checked, they are very similar.

3. The two forms differ in the carpogonial branch. This may be only a case of further parasitic reduction in *H. pachyderma.*

4. The two plants differ in all the details of the nourishment of the ooblastema, the essential mechanism of ooblastema fusion, and the secondary fusions which are so common in *H. pachyderma* are entirely absent in *H. mirabilis.* The two plants are sufficiently distinct to be placed in different genera at least. In the present lack of knowledge of these details in so

many common forms, the nomenclature is purely provisional, and as only three species of the old genus *Choreocolax* have been investigated, it is at least possible that the remaining five figured by Reinsch may in the future prove to be equally distinct. Under the circumstances the genus *Harveyella* may still provisionally include the two distinct forms *H. pachyderma* and *H. mirabilis.*

5. During the year these two parasites pass through the ordinary full Floridean life-cycle twice, in water of ten or more fathoms below low-water mark. This is accompanied from October to April by a development of the same cycle once, at near low-water mark, but in very much greater numbers. From May to October both species entirely disappear from the shallower water.

6. These two species have attained their great similarity of appearance and somatic details partly from reduction due to their parasitic habit. They were reduced from species of Florideae of the present-day type, after these species had reached the full Floridean life-cycle.

In conclusion my thanks are due to Dr. A. H. Church, of Oxford, for very helpful criticism and suggestions in the final preparation of this paper.

The drawings were made in outline by means of the camera lucida at table level, unnecessary detail afterwards omitted, and the figures in many cases reduced.

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The Anatomy of the Branching Fronds of some Cultivated Varieties of Ferns.

BY

S. WILLIAMS.

With fifty-three Figures in the Text.

INTRODUCTION.

THE branching of Fern fronds, and especially the relation between dichotomous and monopodial branching, is of considerable general interest. Branching varieties, or 'mutations' as Goebel[1] has recently termed them, have been long known, and a number of them are figured in various works of the seventeenth century. In more recent times, the prevalence of forked varieties has been noted by various investigators in their consideration of the problems connected with the architecture of the Fern frond. Bower,[2] Potonié,[3] and others have used such forms in support of the view that the various types of branching of Fern fronds are all derived from a dichotomous type.

The behaviour of the vascular strands during the process of branching has, however, been described in only a few cases. Professor Bower[4] has mentioned a furcate variety of *Nephrodium molle* as exhibiting a very equal division of the vascular supply at the level of branching. An equal division of the strand is also described as occurring in branched fronds of *Platyzoma microphyllum*.[5] Apart from these two cases, however, the anatomy of the numerous furcate varieties does not appear to have been described, and it has been generally assumed that an equal division of the vascular supply takes place at the level of branching in all of them. An examination of some branched fronds of *Scolopendrium vulgare* showed a distinct inequality

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[1] Goebel: Organographie der Pflanzen. Aufl. ii, pp. 1064–70.

[2] Bower: On Leaf Architecture as illuminated by a Study of Pteridophyta. Trans. Roy. Soc., Edin., li.

[3] Potonié: Grundlinien der Pflanzen-Morphologie, pp. 118–20.

[4] Bower; l. c., p. 698.

[5] Thompson: Anatomy and Affinity of *Platyzoma microphyllum*. Trans. Roy. Soc., Edin., li.

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of the supply to the two divisions. The possibility, in this case, of the smaller of the branches being lateral relative to the other rendered it desirable to make a more extended investigation and a number of branching varieties of *Nephrodium*, *Scolopendrium*, *Polypodium*, *Athyrium*, and *Osmunda* have been examined. The anatomy of the varieties of the first three genera will be described rather fully; that of the others will only be mentioned in relation to general conclusions.

The varieties have been grouped together under the species from which they have been derived, and in each case the structure of the vascular strand of the petiole and rachis in the normal frond has been briefly described, as aiding in the understanding of the structure of the branched fronds. Although the nomenclature of the varietal forms is somewhat confused, most of the varieties described in this paper are well-established ones, and the varietal names used are those given by E. J. Lowe in his 'British Ferns'.

NEPHRODIUM FILIX-MAS, Rich.[1]

The structure of the petiolar trace of *Nephrodium Filix-mas* is well known, and need not be described at length since it is similar to that of *Peranema*, of which a detailed account has been given by Davie.[2] In a transverse section at the base of the petiole a horseshoe-shaped curve of strands with the open end of the horseshoe facing towards the adaxial surface is seen. The two adaxial strands are the largest and possess characteristically hooked xylem masses, and it is from the backs of these hooks that the pinna traces are given off extramarginally. The abaxial strands are smaller and each of them possesses a simple plate of xylem. There are also smaller commissural strands linking up the main ones to form a network. In higher regions of the frond the number of strands forming the horseshoe becomes less, and near the tip there is only a single strand present. This strand, from which the last few pinna traces are given off in a marginal fashion, possesses a xylem plate without hooks.

There are numerous varieties of *Nephrodium Filix-mas* which exhibit a branching either proximally or distally in the frond. A number of such varieties have been examined, and the following are described as being representative:

1. Var. *ramo-cristatum*.

The fronds of this variety are characterized by a branched and crested habit, the main branching occurring in the lower part of the frond. There may only be a single proximal branching, or one or both arms of this first branching may divide again. Occasionally a trifurcate frond is found. The frond on which the following description is based had each shank of the first forking again divided.

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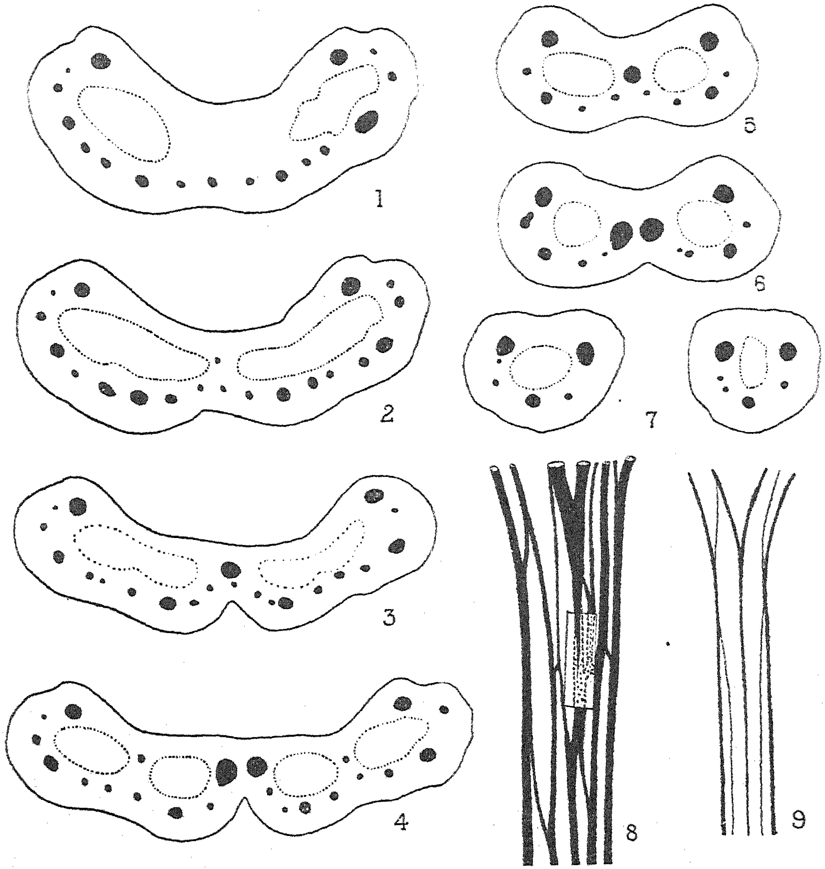
[1] *Dryopteris Filix-mas*, (L.) Schott, of Christensen.

[2] Davie: Structure and Affinity of *Peranema* and *Diacalpe*. Ann. Bot., xxvi, p. 245.

At the base of the petiole the vascular strands are arranged in a some-



FIGS. 1–9. *Nephrodium Filix-mas*, var. *ramo-cristatum*.

FIGS. 1–4. Series of transverse sections of rachis (adaxial surface uppermost) from base to level of branching. × 7. The dotted lines enclose areas of slightly decayed tissue.

FIGS. 5–7. Similar series in one arm of the first branching. × 7.

FIG. 8. Dissection of rachis of adult frond.

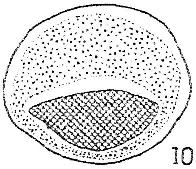
FIG. 9. Dissection of rachis of frond from a two-year-old plant.

what flattened horseshoe curve, the terminal members of which possess hooked xylem masses as in the case of the normal frond (Fig. 1). At a level a little distance below the branching a new strand appears in a central position in the concavity of the horseshoe and quite separate from the other strands of the curve (Fig. 2). It is this central strand which is of primary importance in the division of the vascular supply which accompanies the branching. At successively higher levels in the rachis it is seen to increase gradually in size, remaining free for a considerable distance but anastomosing with the adjacent abaxial strands at higher levels (Fig. 3). As the level of branching is approached, the central strand divides into two, so that there now become evident two horseshoe curves of strands (Fig. 4) which pass into the two shanks of the branching. At a level a little below the actual forking, a further complication arises in connexion with the further branching of each shank. At this level the rachis is already bilobed, and, in a central position in each of these lobes, there arises a small strand similar to the main central strand (Fig. 4). These strands maintain their central position in the lobes, gradually increasing in size and ultimately passing into the arms of the branching. In each arm the central strand behaves in an exactly similar manner to the main central strand, and by its division gives rise to the inner, adaxial corner strands of the second branching (Figs. 5–7). In this manner, each of the four shanks resulting from the two forkings comes to be essentially similar in construction to the rachis of a normal, unbranched frond.

The general relation of the strands is shown in Fig. 8, which is a camera lucida drawing of a dissection of the petiole of a frond which showed only a single branching. A small piece of paper was inserted under the end of the central bundle to indicate its separateness from the strands of the horseshoe, the course of the strands behind this paper being indicated by dotted lines.

The details of the elaboration of the central strand, as seen in transverse sections from below upwards, are shown in Figs. 10–14. A section immediately above the level where the strand first appears shows a small group of tracheides surrounded by phloem and endodermis (Fig. 10). At a higher level a small group of tracheides appears to the adaxial side of the main xylem mass (Fig. 11), and at successively higher levels this adaxial group enlarges and becomes extended in a direction parallel to the adaxial surface of the rachis (Fig. 12). Simultaneously with these latter changes the abaxial xylem mass enlarges, and in a central position in this there becomes evident an island of phloem. At still higher levels the adaxial xylem mass becomes connected to the abaxial mass; the phloem island increases in size, and finally breaks through to the abaxial side (Fig. 13). At the level of branching this strand divides to give two strands which continue as the inner adaxial strands of the two arms of the branching (Fig. 14).

A small trifurcate frond of this variety was also examined, and the type of vascular division (illustrated in Figs. 15–18) was found to be similar to that described above, the trifurcate appearance being due to the fact that one arm of the first branching divided again almost immediately. Two central strands (*a* and *b* in Fig. 16) are present in the concavity of the horseshoe, and these, by their enlargement and division at levels approaching



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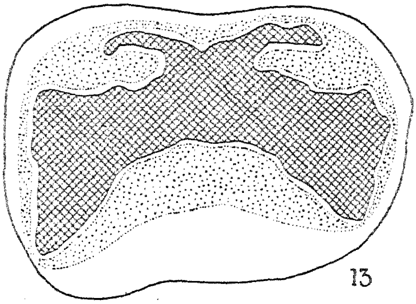

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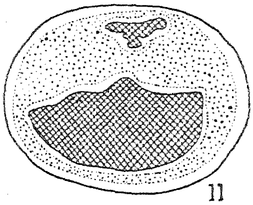

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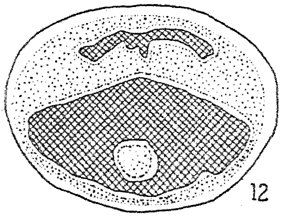

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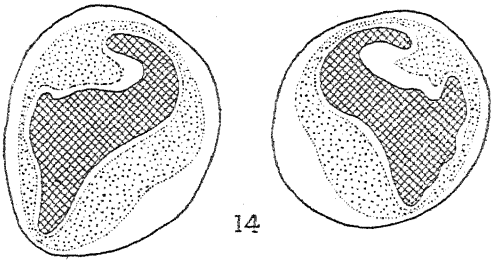

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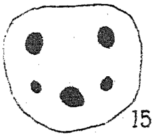

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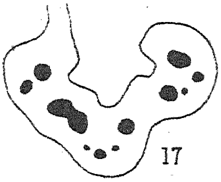

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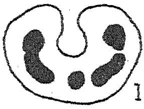

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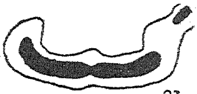

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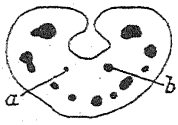

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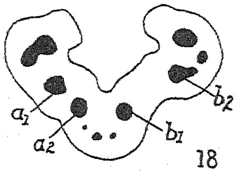

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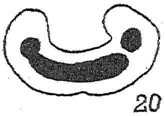

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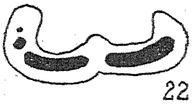

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FIGS. 10–18. *Nephrodium Filix-mas*, var. *ramo-cristatum*.

FIGS. 10–14. Series of transverse sections of the central strand from its origin up to the level of branching; xylem, cross-hatched; phloem, stippled; endodermis, plain line. × 60.

FIGS. 15–18. Series of transverse sections of rachis of trifurcate frond from its base to just below the branching. × 9.

FIGS. 19–22. *Nephrodium Filix-mas*, var. *polydactylum* (Dadds). Series of transverse sections in the region of branching. × 9.

the branching, give rise to the adaxial strands labelled *a 1*, *a 2*, *b 1*, and *b 2* in Fig. 18.

The fronds of young plants of this variety also show a branching habit, though the branching is more irregular than in fronds of adult plants. In the fronds of two-year-old plants there is no special central strand, but the central abaxial strand becomes larger at levels approaching the branching, and at the actual branching divides to form the inner adaxial strands of the two arms. A dissection of the petiole of such a frond is shown in Fig. 9. In the fronds of one-year-old plants the type of vascular division is even simpler. At the level of branching there is a single strand, with a simple xylem plate, which divides to supply the two shanks.

In addition to the proximal branchings so far described, the fronds of this variety are also branched in a distal region to give a crested appearance. Such distal branchings are met with in a large number of varieties and the vascular division which accompanies them is of a simpler type than that described for the proximal branchings. The structure seen in the variety *polydactylum* (Dadds) is typical of such distal forkings and will now be described.

2. Var. *polydactylum* (Dadds).

The branchings of this variety all occur in a distal region of the frond. There is usually a bifurcation of the rachis, and in each resulting shank further branchings occur to give the polydactylous appearance.

The configuration of the vascular supply up to a level a little below the bifurcation is essentially similar to that seen at a corresponding level in a normal unbranched rachis, consisting, in the fronds examined, of a horseshoe curve of four or five strands. At levels approaching the branching these strands coalesce, so that immediately below the branching there is seen a flattened curve of vascular tissue without gaps and with ill-defined hooks at each extremity of the xylem mass. This solid arch divides equally to supply the two shanks of the branching (Figs. 19–22). Each daughter trace has its xylem mass in the form of a slightly curved plate with a small, indistinct hook at each end. In the further branchings which occur in each arm there is a simple division of the single vascular strands.

The type of structure found in the two varieties described above holds also for several other varieties which have been examined. In the fronds of adult plants of all of them, a distinction can be drawn between proximal and distal branchings, the former being characterized by the central strand type of division, and the latter by a simple division of a solid, much-flattened curve of vascular tissue. The type of structure present in the fronds of young plants of *N. f.-m.* var. *ramo-cristatum* stands in evident relation to the simpler vascular skeleton of fronds of that age. The fronds of no other young plants have been examined.

SCOLOPENDRIUM VULGARE, Sm.[1]

The structure of the vascular strands of the normal rachis of *Scolopendrium vulgare* has been described by Luerssen,[2] Bertrand and Cornaille,[3] and, so far as its phylogenetic significance is concerned, by Bower.[4] The varying conformation of the strand at different levels in the frond has not received much attention and a brief statement of the facts may therefore be given.

At the base of the rachis there are two strands, each possessing a strap-shaped xylem mass surrounded by phloem, pericycle, and endodermis. At higher levels the two strands move together and at a level just below the laminal portion unite to form a single strand with an X-shaped xylem mass (cf. Fig. 23). The pinna traces are given off from the adaxial arms of the xylem mass in a marginal fashion. In the distal region of the frond the strand appears as a simple plate, the abaxial arms of the xylem mass being no longer present.

Scolopendrium vulgare is particularly rich in varietal forms, many of which possess branched fronds. In a consideration of the anatomy of such branched varieties the main interest lies in the modifications of the X-strand at levels below the branching. A number of varieties have been examined, and the following are selected for description as illustrating the various types of branching met with in this group:

1. Var. *ramo-cristatum*.

As the name indicates, the fronds of this variety are both branched and crested. Figs. 23–9 illustrate the structure of a frond in which the main branching occurred just below the laminal portion. Further branchings occurred in each shank of this first forking, and in the distal region of the frond repeated branchings gave a heavily crested appearance.

There is present near the base of the petiole an X-shaped strand which is similar to the strand of a normal petiole (Fig. 23). Figs. 24–6 illustrate the changes in the conformation of the strand at levels approaching the first branching. These changes result in the formation of what may be termed a double normal strand at a level just below the branching (Fig. 26). This strand is laterally extended and possesses a xylem mass which consists of two X-shaped masses united by their adaxial arms, and at the actual branching it divides to give two strands, each of which closely resembles the strand seen at the base of the petiole.

Immediately above this first branching one arm again divides, and

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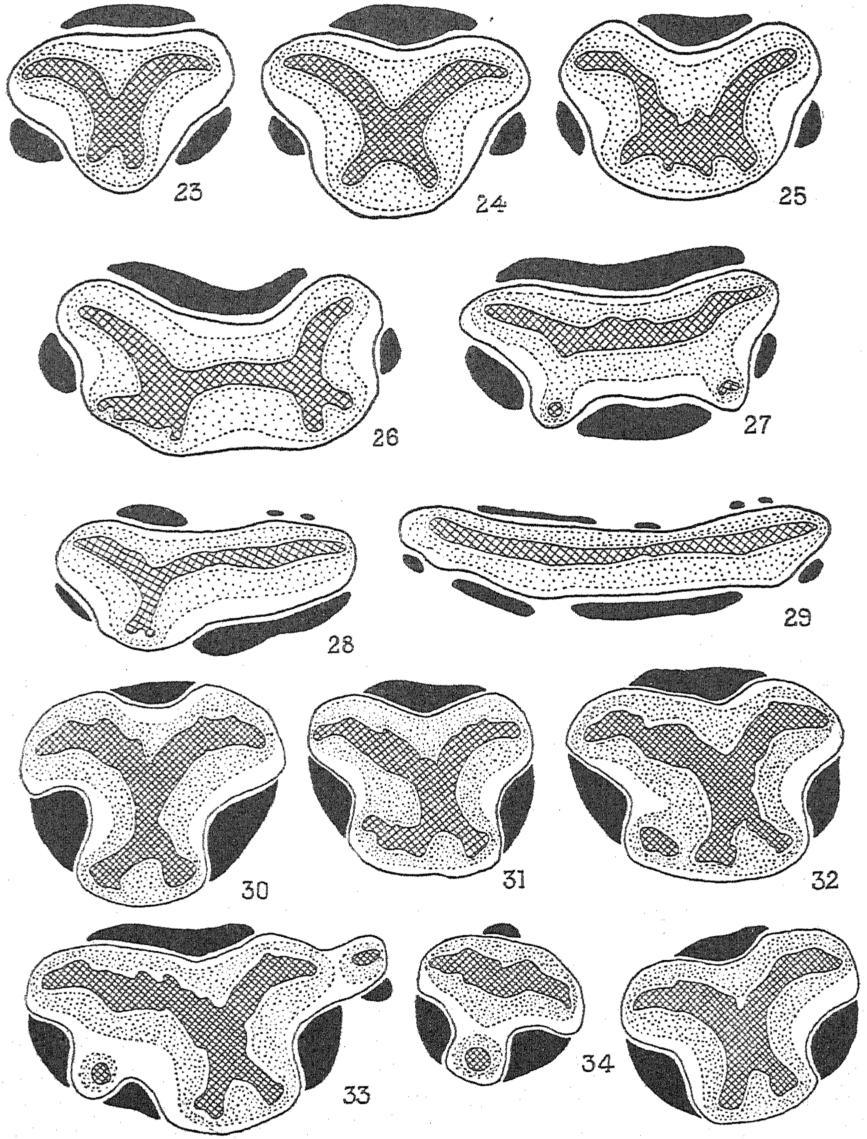
[1] The *Phyllitis Scolopendrium*, (L.) Newm., of Christensen.

[2] Luerssen: Die Farnpflanzen, p. 120.

[3] Bertrand and Cornaille: Étude sur quelques caractéristiques de la structure des Filicinées actuelles.

[4] Bower: Studies in the Phylogeny of the Filicales. Ann. Bot., xxviii, p. 411.



FIGS. 23–9. *Scolopendrium vulgare*, var. *ramo-cristatum*.

FIGS. 23–6. Series of transverse sections of the vascular strand from below up to the level of the first branching. × 60.

FIG. 27. Transverse section at level of branching in one arm of the first branching. × 60.

FIG. 28. Transverse section at level of branching in the other arm. × 60.

FIG. 29. Transverse section at level of one of the distal branchings. × 60.

FIGS. 30–4. Another form of *Scolopendrium vulgare*, var. *ramo-cristatum*. Series of transverse sections up to the level of branching. × 60.

In Figs. 23–50 the xylem is cross-hatched; the phloem, stippled; the endodermis, a plain line; masses of sclerenchyma, black.

Fig. 27 illustrates the structure of the strand just below this second branching. The division of this strand is equal, but each daughter trace, instead of possessing an X-shaped xylem mass, has only an adaxial plate of xylem together with a small isolated group of abaxial elements.

A second branching also occurs in the other arm of the first forking and in this case the branching is very unequal. Fig. 28 illustrates the structure of the strand just below the level of branching, the xylem mass here consisting of an X-shaped mass together with a lateral extension of adaxial xylem. By the division of this strand the larger arm is supplied with a strand possessing an X-shaped xylem mass, and the smaller one with a strand possessing only an adaxial plate of xylem.

In the distal regions of all branches of the fronds the strands are devoid of abaxial xylem and appear as laterally extended plates. Fig. 29 illustrates one such distal branching where the xylem plate is dividing unequally.

2. Another form of var. *ramo-cristatum.*

This is the variety which was first examined and which is mentioned in the introduction as showing an unequal division of the petiolar strand at the level of branching. It closely resembles the plant of the variety *ramo-cristatum* just described, and would probably fall under this name; its fronds are much larger than those of the plant described under 1 above. The petiole is usually branched in a proximal region and further branchings occur at higher levels in the frond. Frequent branchings in a distal position give a crested appearance to the fronds.

The changes in conformation of the X-strand from below upwards to the level of branching are illustrated in Figs. 30–4. As a result of these changes, the strand at a level just below the branching has the conformation shown in Fig. 33. One arm of the branching is supplied with a normal X-strand, while the strand supplying the other arm possesses an adaxial plate of xylem together with an isolated mass of abaxial xylem (Fig. 34). At a higher level the strand of the smaller arm again comes to possess an X-shaped xylem mass by the appearance of tracheides between the adaxial plate and the abaxial group.

The distal branchings of this variety exhibit the same simple type of vascular division as those of the var. *ramo-cristatum* (cf. Fig. 29).

3. Var. *grandiceps.*

The frond on which Figs. 35–9 are based was not only crested but showed an equal branching of the rachis some distance below the laminal portion.

As a result of a series of changes in the X-strand at levels approaching the branching (Figs. 35–7) there is present immediately below the branching a laterally extended strand possessing only adaxial xylem (Fig. 38). At the actual branching each arm is supplied by a strand possessing a grooved plate of adaxial xylem (Fig. 39). As in the varieties previously described, the distal branchings show a simple division of a vascular plate.

4. Var. *curiosum.*

The fronds of this variety exhibit varying degrees of branching, and there is also a considerable variation as regards the position of branching in the frond. The laminae are very much reduced, being represented in the greater part of the length of the frond by a narrow wing on either side the mid-rib, but extending in the distal portion to form a fan-shaped blade. In the case of the frond on which Figs. 40–2 are based there was only a single branching, which occurred about half-way up the length of the frond.

Figs. 40–2 illustrate the structure of the petiolar strand at levels approaching the branching. The most marked feature is the cutting into the central xylem mass from the adaxial side by a wedge of phloem, with the result that immediately below the branching the strand comes to have the curious configuration shown in Fig. 42. By the division of this strand each shank of the branching is supplied by an X-strand, which differs from the normal type in the smallness of its adaxial arms.

Several other varieties examined show essentially the same features as those described above. All the distal branchings exhibit the same simple type of branching as that described for the variety *ramo-cristatum.* The conformation of the strand at the level of the proximal branchings shows considerable variation. It is thought that the central type is that which shows a double X-strand at the level of branching, and that the other types are modifications of this, due to variations in the amount of abaxial xylem present in one or both of the daughter strands. Figs. 26, 33, 28, 27, and 39 show a progressive and gradual reduction of the double X-strand.

POLYPODIUM VULGARE, L.

The petiolar strand of *P. vulgare* has been described by Luerssen,[1] Davie,[2] and others. The main facts (which have been verified) are as follows:

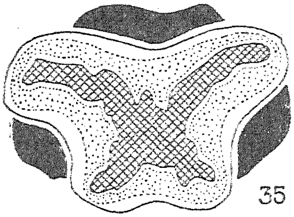
A section near the base of the petiole shows two main strands in an adaxial position together with a varying number of smaller abaxial strands (cf. Fig. 43). At higher levels in the petiole the strands move together until, at a level just below the laminal portion, there is usually only a single strand present. The xylem mass of this strand is V-shaped or Y-shaped according to the amount of abaxial xylem present, and is surrounded by phloem, pericycle, and endodermis. From the adaxial arms of the xylem

[1] Luerssen: l. c., p. 53.

[2] Davie: The Leaf-trace in some Pinnate Leaves. Trans. Roy. Soc., Edin., vol. lii, p. 8.

pinna traces are nipped off in a typically marginal fashion. In distal regions of the frond the strand decreases in size and in the terminal portion is seen as a flat plate.

A number of branching varieties of *Polypodium* have been examined, and, as in the case of *Scolopendrium*, the branchings may be divided into two groups, viz. those occurring in proximal and those in distal regions of the frond. The following varieties are described as being representative: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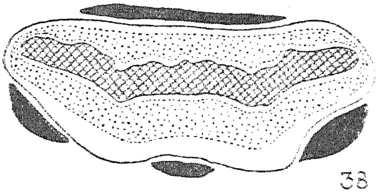
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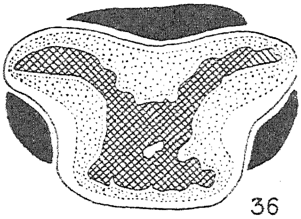
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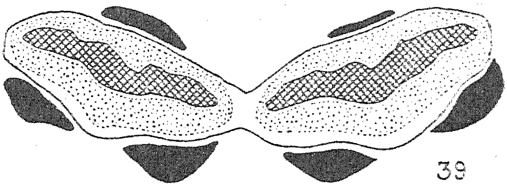
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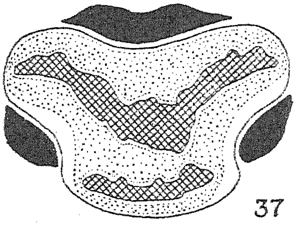
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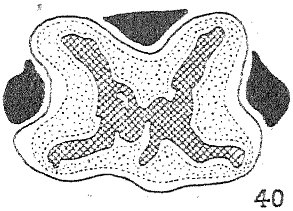
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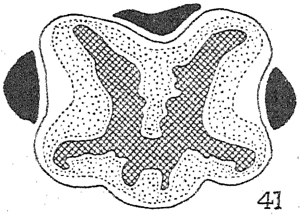
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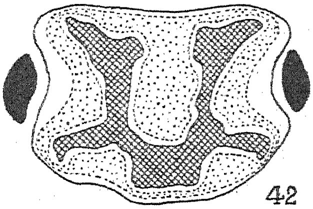
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FIGS. 35–9. *Scolopendrium vulgare*, var. *grandiceps*. Series of transverse sections of the vascular strand up to the level of branching. × 60.

FIGS. 40–2. *Scolopendrium vulgare*, var. *curiosum*. Series of transverse sections of the vascular strand in the region of branching. × 60.

1. Var. *ramosum*.

The fronds of this variety usually show a single main branching low down in the frond. Figs. 43–7 illustrate the vascular structure from the base up to the level of branching in a frond where the branching was slightly unequal. The three strands which are present at the base of the rachis unite at the base of the laminal region to give a single strand with a Y-shaped xylem mass. At levels approaching the branching one of the adaxial arms of the Y becomes laterally extended (Fig. 46), and at the actual branching the larger arm is supplied with a strand possessing a Y-shaped xylem mass, while the smaller one is supplied with a strand possessing only an adaxial plate of xylem (Fig. 47).

Other fronds, however, exhibit an equal division of the vascular tissue, there being present at the level of branching a double normal strand.

A number of varieties with fronds branched in the proximal region exhibit essentially the same features as those described above.

2. Var. *grandiceps* (Forster).

All the branchings are in a distal region, the fronds being heavily crested. In the lower regions of the fronds the structure of the petiolar strand is similar to that in the normal frond. Below the branching the strand has already lost the abaxial tongue of xylem and so appears as a laterally extended flat plate. Figs. 48 and 49 illustrate how, at the level of branching, this strand divides in a simple manner to supply the two arms of the first branching. In the arms of the branching the strands persist as simple plates, and in the further numerous branchings which give the large crested head they divide in a simple manner, though often unequally, since there is frequently a sympodial development of the branches in terminal regions of the frond. Fig. 50 illustrates the division of the vascular plate in one such terminal branching.

The branching varieties of *P. vulgare* thus show characters similar to those already described in the case of *Scolopendrium* varieties. In the proximal branchings it has been seen that branching may involve either an equal or unequal division of the petiolar strand, but it is thought that the central type is that showing a double normal strand at the level of branching. The cases where the division is unequal may be thought of here, just as in the *Scolopendrium* varieties, as a modification of the central type due to differences in the amount of abaxial xylem in the daughter strands. The distal branchings all show a simple division of the vascular plate which is present in the terminal regions of the frond.

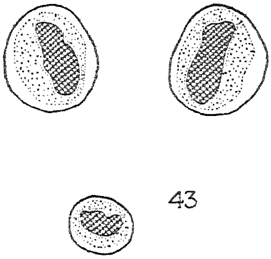
CONCLUSION.

In the preceding sections the facts have been given without any reference to the general problem presented by the architecture of the Fern leaf. An attempt may now be made to indicate briefly the bearing of the facts on this problem, which has already received a considerable amount of attention from various investigators.[1]

---

[1] See Introduction.

In all the varieties examined it is possible to divide the branchings into two categories according as to whether the branching occurs in a proximal or a distal region of the frond. The proximal branchings are the more interesting inasmuch as they present a considerable amount of variation in the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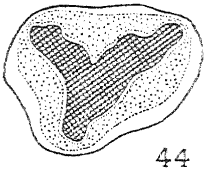
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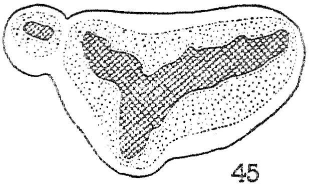
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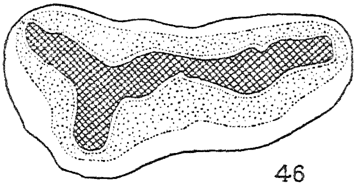
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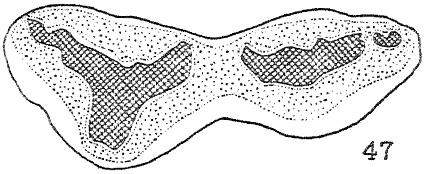
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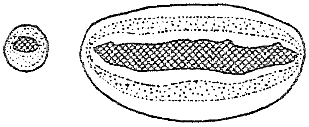
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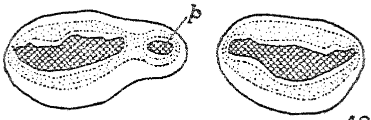

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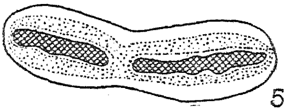
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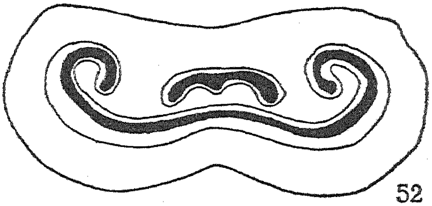
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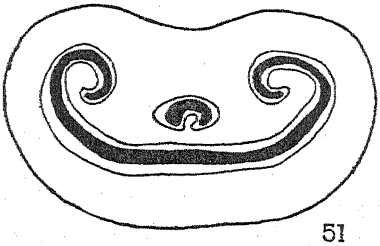
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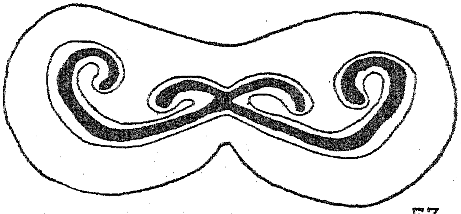
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FIGS. 43–7. *Polypodium vulgare*, var. *ramosum*. Series of transverse sections of the vascular strand up to the level of branching. × 60.

FIGS. 48–50. *Polypodium vulgare*, var. *grandiceps* (Forster).

FIGS. 48–9. Transverse sections at level of main branching. × 60. *p*, a pinna trace.

FIG. 50. Transverse section at level of one of the terminal branchings. × 60.

FIGS. 51–3. *Osmunda regalis*, var. *ramo-cristatum*. Series of transverse sections of the rachis from below up to the level of branching. × 9.

method of division of the vascular supply, and these will be considered first.

It has been suggested that the central type of vascular division in the proximal branchings is that which shows a double normal strand at the level of branching, the division of the vascular supply being perfectly equal. This is very clearly shown in the case of the *Nephrodium* varieties, where a double horseshoe curve of strands is present at the level of branching. In the case of branching fronds of *Asplenium bulbiferum*, and a branching variety of *Athyrium Filix foemina*, an equal division of the vascular supply was also met with, there being a double X-strand in the former, and a double V-strand in the latter, at the level of branching. Branching varieties of *Osmunda regalis* also show this type of branching very clearly, and just below the branching a double C-strand is present (Figs. 51–3).[1] The branching in all these cases is clearly dichotomous.[2]

The proximal branchings in the varieties of *Scolopendrium vulgare* and *Polypodium vulgare* are not so easily understood, since they exhibit a wide range of variation in the conformation of the strand at the level of branching. These variations may be thought of as modifications of the type showing a double normal strand, resulting from variations in the amount of abaxial xylem present in one, or both, of the daughter strands. The branchings in these varieties must then be regarded as being essentially dichotomous, though the shanks of the dichotomies are often unequal.

In addition to the above interpretation of the anatomical construction, the morphology of the branched fronds makes it difficult to regard any of the branches as being lateral and homologous with a pinna. Branching often occurs below the laminal portion, and, in the case of branchings occurring in the laminal portion, the smaller of the branches does not appear to take the place of a pinna.

It must therefore be concluded that the balance of the anatomical and morphological evidence lies in favour of the view that the proximal branchings are all dichotomies, though the shanks may, in certain cases, be unequally developed.

The case of the distal branchings is somewhat simpler in relation to the simpler conformation of the normal strand in these regions. These branchings are all of a dichotomous nature, though there is often a considerable degree of sympodial development of the shanks.

In the absence of any knowledge of the development of the frond in branching varieties, it would seem justifiable, on the grounds stated above, to regard all the branchings observed as being essentially dichotomous. This conclusion confirms the assumptions which have been made previously that the frequent abnormal branchings of Fern fronds are dichotomies; but it has to be further recognized that varying degrees of sympodial development may occur.

---

[1] The central strand type of vascular division present in *O. regalis* varieties has been described above as occurring also in *Nephrodium Filix-mas*, var. *ramo-cristatum*. In both cases the central strand, by its enlargement and division, forms the inner adaxial portions of the two daughter strands.

[2] The term 'dichotomy' as used in this paper does not necessarily imply an equal division of an apical cell, but is used rather in the sense of the 'apparent dichotomy' defined by Bower as 'the continuation of one shoot by two equally strong ones'.

This frequent abnormal dichotomy falls into line with the rest of the evidence which has been brought forward from the normal configuration of living and extinct species, notably by Bower and Potonié, in support of the dichotomous theory of frond construction, which holds that the modern Fern frond with its typical monopodial construction was derived from a dichotomously branched structure.

SUMMARY.

1. The anatomy of the branched fronds of a number of varietal forms of leptosporangiate Ferns has been described with special reference to the method of vascular division.

2. The branchings are divided into two groups: those occurring in a proximal and those in a distal region of the frond.

3. It is shown that a double normal strand is the characteristic structure present at the level of branching in proximal branchings, though this may be modified in various ways.

4. The distal branchings all show a simple division of a laterally extended vascular plate.

5. All the branchings are held to be dichotomous, though there may be a certain amount of sympodial development of the shanks.

6. The relation of these facts to theories of frond construction is briefly discussed.

In conclusion, I wish to express my thanks to Professor W. H. Lang for suggesting this investigation and for much helpful criticism throughout its progress.

CRYPTOGAMIC RESEARCH LABORATORY,
BOTANICAL DEPARTMENT,
MANCHESTER UNIVERSITY.

Studies of the Rate of Growth of Leaves by a Photographic Method.

I. The Determinants of the Rate of Growth of First Leaves of Phaseolusvulgaris.

BY

M. C. VYVYAN

(*Gonville and Caius College Frank Smart Student in Botany*).

With five Figures and nine Graphs in the Text.

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INTRODUCTION.

MUCH of the past work on the rate of increase in area of leaves seems to have suffered somewhat from the lack of a simple and accurate method of ascertaining the area of a leaf without damaging the leaf or plant. The methods usually employed may be classified under two main types: either the area is calculated from certain measurements made on the surface of the leaf, or else it is obtained direct by means of a planimeter. The latter type of method is the more accurate of the two, since it is not affected by variations in the shape of the leaves, but it suffers from a serious disadvantage—it involves the destruction of the leaves; no two successive measurements can therefore be made from the same leaf. Gregory, in his recent paper (8) on *Cucumis sativus*, has made use of a method of the first type; he estimated the area of the leaves day by day by means of formulae based upon the relationship of the area of the leaf to certain measurements made on its surface. But, in spite of the time and trouble such a method must entail, he only hoped to obtain results significant to five per cent. It is obvious that the accuracy of such a method must largely depend upon the degree to which the leaves maintain their shape unchanged during growth; Gregory found that the leaves of *Cucumis sativus* changed with increasing age from irregular hexagons to irregular octagons, and he had to use different measurements and different formulae according as the leaf approximated to the one shape or to the other.

A method of the second type has been used by Briggs, Kidd, and West (3, 4, and 10) in their recent studies of growth in *Helianthus*; they cut off the leaves, made tracings from them, and determined the area of the tracings by means of a planimeter. To discover the rate of increase in leaf area from week to week, they had to use a different batch of plants for every week; had it been necessary to make determinations every day, the number of plants required would have been inconveniently large. For their purposes, however, this method left little to be desired, because these authors were studying the relationship of the increase in the area of the leaves to the true growth of the plant, as expressed by its increase in dry weight. Although these authors have shown, in the papers mentioned above, that increase in leaf area cannot safely be taken as a measure of the true growth of the plant, which can only be found from the changes in dry weight, nevertheless it is a form of growth, i. e. a growth in area, and as such it is worthy of study. Provided its necessary limitations are not lost sight of, a study of increase in leaf area may throw light on many problems that could not easily be attacked by the more fundamental dry weight method. For example, exact quantitative data are almost completely lacking in the case of the compensative changes in the rate of increase in area that are known to occur in certain leaves, when certain other parts of the plant are removed. To carry out a study of this nature, a method is required that combines the advantages of both types of method mentioned above; it must be possible to make successive measurements of the area of the same leaf, and to make them with the accuracy that can only be obtained by the use of a planimeter. Theoretically it should be possible to make tracings from the leaves without cutting them off, but in practice this is tedious and difficult; the present writer has therefore devised a photographic method which will be described below. To test the applicability of the method, a number of experiments of a preliminary nature were carried out in 1921 on the dwarf kidney bean (*Phaseolus vulgaris*), and in the following year a systematic study of the increase in area of the first leaves of that plant was begun; the results of some of the experiments in this study will form the subject of this paper.

I wish at the outset to express my gratitude to Dr. F. F. Blackman for his unfailing advice and helpful criticism during all stages of the work, and to Mr. Udny Yule for assistance in devising a method of ascertaining the influence of the varying external conditions upon the rate of growth.

METHODS.

The method adopted was as follows: The plants were grown in pots and were taken in groups of four to a dark room at approximately the same time of day every day, where photographic prints were made of the leaves on 'Slogas' gaslight paper; the actual leaves being used as negatives without detaching them from the plant. No plant was in the dark room for more than about a quarter of an hour. With reasonable care this process can be carried out without in any way damaging the leaf or plant. Beneath the leaf was placed a piece of felt with a slot to take the stalk, beneath the felt was a board with a similar slot; the printing paper was inserted between the felt and the under surface of the leaf, which was pressed gently on to the paper by means of a sheet of glass. At first, weak spring clips were used to keep the glass in position, but, later, it was found preferable to use slight pressure between finger and thumb. After exposure and development, the result was an exact image of the leaf in white on a black background. The outline of this image was followed with a planimeter, and the exact area thus obtained of the leaf at the time when the print was made. As the veins of the leaf in this plant are more transparent than the rest of the lamina, their position could be obtained, when required, by slightly increasing the exposure when making the print; they showed then as thin black lines in their correct positions on the finished print. When dealing with thirty plants, the process of printing took about a couple of hours, but, as the process was always begun at approximately the same hour of the day and the plants always dealt with in the same serial order, the variation in the times at which the prints were made from the same leaf on different days was never more than about half an hour. At the time the print was made, the serial number of the plant and of the leaf, the date and the exact hour and minute at which it was taken, were written on its back. The prints were developed later in bulk, and after fixation and washing were dried on ferrotype plates.

Possible sources of error. It is obviously necessary to use leaves that present a flat surface: when they fall into folds it is possible to obtain the area of the folds by slight over-exposure, for the region of the fold then shows whiter than the rest of the image on the print. But, as pressure under the glass tends in time to make the leaf split along the edges of the fold, it is better to reject all such leaves at the start.

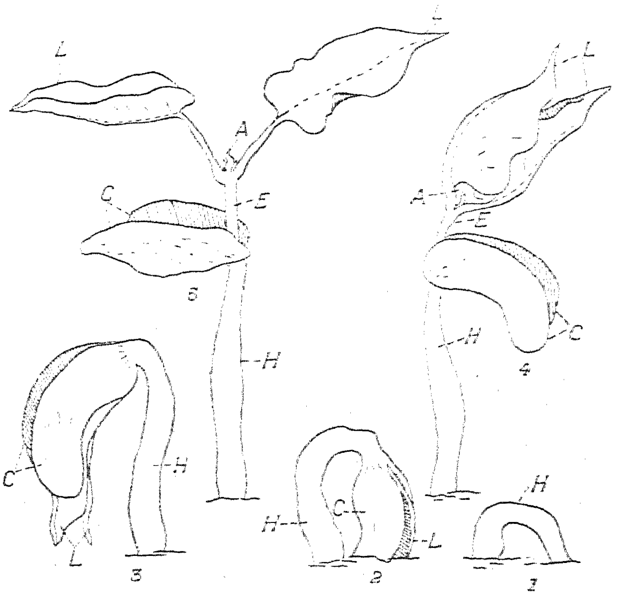
There was another possible source of error—the prints might stretch or shrink during the process of development or drying; to determine the magnitude of this source of error a test was made. A negative was made from transparent millimetre paper, and a number of prints made from the negative and treated in the same method as those from the leaves. The squares on the prints were then measured against those on the negative, and the error was found to be well under one-half per cent. As the error of planimetering can be again taken as about one-half per cent., this method of recording leaf areas may be counted upon to give results significant to about one per cent.

There are two other possible sources of error that might affect the results: (*a*) the pressure on the leaf during printing might have a stimulating effect on growth, and (*b*) the mutilation of the plant, through the removal of the apical bud and other parts in the course of the experiments, might lead to a wound-stimulus. As regards the first (*a*), since all the leaves received the same treatment in the process of printing, all may be expected to have been stimulated to much the same extent; moreover, in the case of certain preliminary experiments, there was, in some cases, an interval of two days between the making of successive prints from the same leaf; the total percentage growth in the two days was found to be the same in these leaves as in those of other plants of the same batch, from which prints had been made every day. The second class of stimulatory effects (*b*) are unavoidable when investigating the results of the removal of certain portions of the plant; even if Child and Bellamy's method (5 and 6) was adopted, and physiological isolation by regions of low temperature used in place of actual removal of parts of the plant, it would be impossible to be sure that this treatment had not at least as strong a stimulatory effect as that of actual wounding.

MATERIAL.

The plant selected for the experiments was the dwarf kidney bean (*Phaseolus vulgaris*). The seed used in the preliminary experiments was Canadian Wonder obtained from Messrs. Sanders, Cambridge. In the series of experiments carried out in 1922 the seed used was 'Selected Canadian Wonder' obtained from Messrs. Sutton and Sons, Reading. For all the experiments the seeds were carefully graded, and only those of approximately uniform weight utilized.

In the preliminary experiments the seeds were sown directly into the pots, but in the later experiments a different method was adopted; in these experiments the seeds were sown singly in soil in tin tubes 3 inches long by $2\frac{1}{2}$ inches wide, whose bottoms were closed by pieces of brown paper. As soon as the plants showed above ground, they were transplanted into 7-inch pots with as little disturbance to their roots as possible. The tube was placed on a layer of soil in a pot and the paper carefully drawn away; soil was packed lightly round the tube, which could then be drawn out gently, leaving the plant in position in the pot. This method was adopted to economize space in the greenhouse where the plants were grown; to make sure of obtaining some thirty plants of the same age and the same



FIGS. 1–5. H = hypocotyl; C = cotyledons; L = first leaves; E = epicotyl; A = apical bud.

stage of development, it was found necessary to plant at least eighty seeds on the same day, and eighty of these tin tubes took up far less space than eighty pots would have done.

In this variety of *Phaseolus vulgaris*, the first part of the plant to show above ground is a portion of the arched hypocotyl; it was at this stage of development that the plants were transplanted from the tin tubes into pots. The hypocotyl then elongates considerably and straightens, drawing the cotyledons out of the ground, and during and subsequent to this process the epicotyl lengthens slightly and becomes vertical, drawing the leaves from between the cotyledons, which move somewhat apart. The leaves then draw apart from one another, and become horizontal and expanded; the two processes, the drawing apart and the expansion, being more or less simultaneous and occupying from one-half to a whole day. This is the day referred to as that on which the leaves first expanded. Figs. 1–5 show these various stages; the last two figures (4 and 5) show the approximate stage at which the operations were performed in the experiments described in the present paper.

THE PRELIMINARY EXPERIMENTS.

Only brief mention will be made of these experiments, which were carried out in 1921, as their object was more to master a new technique than to elucidate problems. A few facts of interest were, however, established and are perhaps worth recording.

It was found that the two first leaves of the same plant, even when initially of different areas, increase in area at approximately the same daily percentage rate; the ratio that the area of the one bore to that of the other remained approximately constant, even when their actual areas had increased nearly tenfold. Again, in the case of plants of the same age and the same stage in development, it was found that the daily percentage rate of increase in the area of the first leaves was much the same in every plant, provided they were subjected to identical external conditions. In other words, the material was found to be fairly uniform, and the 'scatter', due to variations in the internal regulative conditions in individual plants, sufficiently small to allow the 'mean' obtained from a few plants to be regarded as significant.

In the spring of 1922 a systematic study of growth in the dwarf kidney bean was begun, and of the series of experiments carried out that year, two form the subject of the present paper; further experiments in that series will be described in a later paper. The writer hopes to continue this systematic study and to extend its scope.

EXPERIMENT I.

Effect of removal of one first leaf upon rate of growth of the other.

Object. This experiment was made to find out if compensative growth takes place in one first leaf when the other is removed at an early stage in development.

Treatment of the plants. One of the two first leaves was removed from each of a number of plants the day those leaves expanded, whilst, in control plants of the same age and the same stage in development, both first leaves were left intact. The cotyledons were left intact on all the plants, but the apical buds were removed from all of them the day the first leaves expanded, and all axillary buds likewise removed as soon as they showed; the object of this was to reduce the number of centres of potential leaf increase. As the first leaves did not expand on the same day in all the plants, three series were constituted and begun on successive days; some of the plants in each series were deprived of one first leaf, the others were kept as controls. Prints were made from every intact leaf every day for over a fortnight, the first prints being made in each case just before the operations were performed. The finished prints were planimetered and the daily areas of the leaves thus found.

History. Eighty seeds of between 0·6 and 0·7 grm. each were sown on the 7th February in tubes, as described above, and a number of the young plants showed above ground and were transplanted into pots on the 13th of the same month; on the 15th some more showed above ground and were similarly transplanted. Of the plants transplanted on the 13th the first leaves expanded as follows: in one plant on the 14th, in seven on the 15th, and in eight on the 16th; whilst the first leaves of eight of the plants transplanted on the 15th expanded on the 17th. Series I was composed of eight plants whose leaves expanded, one on the 14th and seven on the 15th; Series II, of the eight plants whose leaves expanded on the 16th; Series III, of the eight whose leaves expanded on the 17th. Half the plants in each series had the apical bud and one first leaf removed; the other plants in each series were the controls, i. e. had the apical bud removed but both first leaves left intact. These operations were performed in Series I on the 15th, in Series II on the 16th, and in Series III on the 17th. Thus there were three series of plants begun on successive dates, 15th, 16th, and 17th of February, each series consisting of two batches of four plants; the plants of one batch in each series had one leaf cut off and one intact, those of the other had both intact. Owing to casualties,[1] however, the figures for the leaves of some plants could not be included when calculating the mean rates of increase in area. The final number of plants, from which these means were obtained, was as follows: Series I, two plants with both leaves intact and four with one removed; Series II and III, each three plants with two leaves and three with one.

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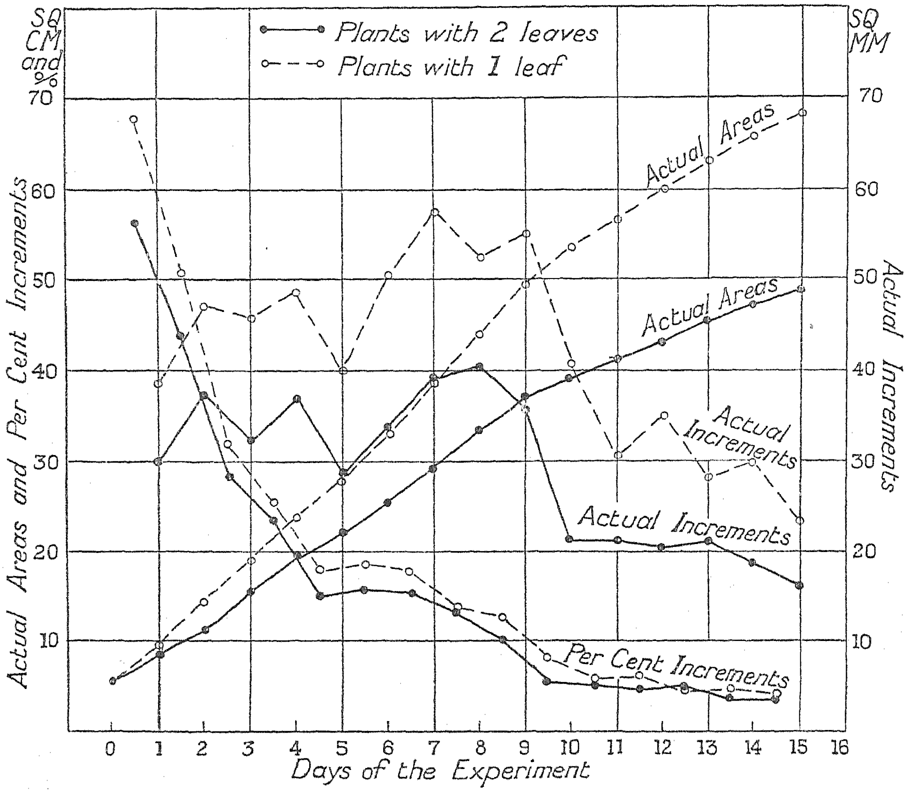
[1] In the batch with two leaves in Series I, the plant whose leaves had expanded on the 14th—the day before the operation—grew at a much slower rate than the other plants in the same batch whose leaves had expanded on the 15th; as this difference was probably due to the plant being at



GRAPH A.

ANALYSIS OF RESULTS OF EXPERIMENT I.

There are three numerical methods by means of which the result of the experiment might be presented. The first method consists in giving the actual areas of the leaves on the successive days of the experiment, as in Table I, where the mean area is given for each of the six batches separately, together with the mean for all the plants with one leaf, and that for all the

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a slightly more advanced stage in development than the others at the time of operation, the figures for this plant were excluded when calculating the mean. From another plant in the same batch one leaf was accidentally broken off the day after the operation. The leaves of two plants in Series II became badly folded and damaged, whilst the cotyledons were accidentally broken off from two plants in Series III.

plants with two; these latter values are plotted on Graph A. This method has the advantage of simplicity, but it does not reveal graphically the true nature of the results. The second method of presenting the results is that of actual daily increments in area; these are also given on Graph A. This method has an obvious disqualification, when leaves of widely different area have to be compared, unless it could be shown that the amount of actual 'growing substance' remained constant, irrespective of the increase in the area of the leaf; but this is not known to be the case, and, on the face of it, seems improbable. The third method is that of presenting the daily percentage rate of increase in area. This is not quite the same thing as the relative leaf growth rate of West, Briggs, and Kidd (10), since the latter is the percentage rate at which the total leaf area of the plant increases. The individual leaves, whose areas will be included in the total leaf area of the plant, will vary from week to week, even if the successive measurements are made on the same plant; for new leaves will unfold from time to time and old leaves may wither away. Under the particular conditions of the present experiment, the total leaf area of the plants was, in point of fact, always made up of the areas of the same individual leaves, since the unfold-

TABLE I.

Mean Areas of Leaves in sq. cm. on Successive Days of the Experiment.

Day of Expt.	*Both Leaves left intact.*			*One Leaf cut off.*			*Both Leaves left intact.*		*One Leaf cut off.*
	Series I. 2 *Plants.*	*Series II.* 3 *Plants.*	*Series III.* 2 *Plants.*	*Series I.* 4 *Plants.*	*Series II.* 3 *Plants.*	*Series III.* 3 *Plants.*	*Mean Total Area 2 Leaves.*	*Mean Area of 1 Leaf.*	*Mean Area of Remaining Leaf.*
0	10·85	9·5	13·0	4·7	6·0	6·8	11·12	5·56	5·83
1	17·35	15·5	18·4	8·05	10·8	10·12	17·08	8·54	9·68
2	27·0	22·0	24·6	14·1	15·0	14·0	24·53	12·27	14·37
3	34·4	29·9	28·7	19·3	20·6	16·9	31·0	15·5	18·93
4	44·05	35·3	35·7	25·9	23·9	21·6	38·35	19·195	23·80
5	48·25	42·0	42·1	29·45	28·2	26·0	44·12	22·06	27·88
6	54·0	47·7	50·7	33·9	32·5	32·4	50·8	25·4	32·93
7	59·95	56·0	60·4	38·5	37·7	39·8	58·78	29·39	38·67
8	67·1	65·6	67·9	43·6	43·9	44·3	66·87	33·435	43·93
9	74·05	73·5	74·5	50·7	48·3	49·3	74·08	37·04	49·43
10	79·4	78·8	76·9	56·7	52·6	51·2	78·37	39·18	53·50
11	83·95	81·3	82·7	60·6	53·7	55·4	82·65	41·325	56·57
12	85·05	86·5	88·8	62·65	57·0	60·5	86·78	43·39	60·05
13	88·75	92·2	92·1	65·8	61·0	61·9	91·02	45·51	62·90
14	93·6	95·2	95·7	70·0	62·7	64·9	94·83	47·42	65·87
15	95·35	98·8	100·2	70·8	65·5	68·3	98·12	49·06	68·20
16	97·4	101·3	—	73·8	68·2	—	—	—	—
17	—	—	—	76·6	—	—	—	—	—

The figures for Series I, II, and III, in the case of plants with both leaves left intact, are those for the two leaves taken together, i. e. the total leaf area of the plants. The '0' or zero day of the experiment was the 15th of February in the case of Series I, the 16th of February in Series II, and the 17th in Series III.

ing of new leaves was prevented by the removal of all developing buds; but even in this special case it would be manifestly improper to regard this increase in the area of individual leaves as something of the same nature as the increase in total leaf area in a plant under normal conditions. As in the case of the relative growth rate and relative leaf growth rate of the above-mentioned authors, the daily percentage increments in area may be calculated at compound or at simple interest; but these authors (Briggs, Kidd, and West (3)) have pointed out that the more complicated compound interest 'does not achieve accuracy, as it rests on the assumption that the rate remains constant during the week' (during the day in our case), 'an assumption manifestly incorrect, since the rate varies from week to week. Both methods are purely conventional and only approximate to accuracy, and nothing definite is gained by adopting the more complicated procedure.' The daily percentage increments in area have therefore been worked out at simple interest by means of the formula $\frac{A_2-A_1}{A_1}=\frac{R}{100}$, where A_1 is the area at the begining of the period of twenty-four hours, A_2 the area at the end of the period, and R is the percentage increment in area. The original data of mean daily percentage increments in area, obtained from the six batches of plants, are given in Table II; in order to give each plant in the batch the same 'weight' in the mean, irrespective of the slight initial

TABLE II.

Original Data, percentage Increments in Area.

Date on which 24-hour Period ended.	*Series I.*		*Series II.*		*Series III.*		*Successive Period of 24 Hours since Operation.*	*Mean for all Plants with 2 Leaves.*	*Mean for all Plants with 1 Leaf.*
	2 *Leaves.* 2 *Plants.*	1 *Leaf.* 4 *Plants.*	2 *Leaves.* 3 *Plants.*	1 *Leaf.* 3 *Plants.*	2 *Leaves.* 3 *Plants.*	1 *Leaf.* 3 *Plants.*			
Feb.									
16	59·5	72·6	—	—	—	—	1	56·23	67·60
17	53·5	76·0	66·7	79·7	—	—	2	43·90	50·47
18	27·5	37·25	42·7	38·7	42·5	50·5	3	28·43	32·08
19	28·0	32·25	41·0	38·0	33·5	36·7	4	23·40	25·45
20	9·25	13·9	18·2	16·8	16·8	21·0	5	15·18	17·70
21	12·25	15·1	18·3	18·7	24·0	27·3	6	15·88	18·67
22	10·75	13·75	14·7	16·2	18·0	20·5	7	15·25	17·82
23	12·0	13·0	15·8	17·0	20·7	24·7	8	13·27	13·70
24	10·7	16·25	15·3	16·8	19·2	22·7	9	10·32	12·75
25	7·5	12·0	10·5	10·7	12·5	11·3	10	5·90	8·23
26	5·75	6·8	7·2	9·0	7·75	11·3	11	5·18	5·70
27	1·35	3·5	2·1	2·0	3·0	3·7	12	4·62	6·37
28	4·25	5·0	4·8	6·3	7·7	8·3	13	5·17	4·80
March									
1	5·75	6·4	7·5	7·1	7·7	9·3	14	3·95	4·67
2	1·85	1·6	2·1	2·9	3·75	2·3	15	3·58	3·80
3	2·2	3·05	4·2	4·5	4·0	4·7	—	—	—
4	—	3·8	2·5	4·0	4·7	5·3	—	—	—

differences in the size of their leaves, these mean values were calculated by dividing the sum of the percentage increments of all the plants in the batch by the number of plants in the batch and not direct from the sum of their actual daily areas. The mean percentage increments in area during each of the successive periods of twenty-four hours after the operation, for all the plants with one leaf and for all those with two, are likewise given in Table II, and are plotted on Graph A; on this graph, therefore, the original data are presented in all three different ways.

If the three sets of curves plotted on Graph A be examined, certain facts will become apparent. In the first case, the curves for the plants deprived of one leaf are in each case higher during the whole, or nearly the whole period, than those for the control plants with both leaves left intact; thus the two curves of actual areas diverge, until at the end of fifteen days the mean area of the single leaf is 68·20 in the case where only one is left intact, and is only 49·06 where both are left intact; thus the former has attained an area about 40 per cent. larger than that attained by the latter. In other words, considerable compensative growth has taken place, but not sufficient to make up for the loss of the leaf removed. Turning to the curves of actual increments in area, it will be observed that the curve for the plants with only one leaf is consistently higher, during the whole period, than that for the control plants with both leaves left intact. The same holds good, during the greater part of the period, in the case of the curves of percentage increments in area, but in the case of these latter curves the three series did not yield quite consistent results, as the figures in Table II show; on the first day after the operation, the leaves of the plants where only one was left intact grew faster than those of the controls in all three series, and in the case of Series I and III this higher rate of growth was maintained for over a week, but in Series II, on the other hand, the rate of growth in the plants with only one leaf intact fell on the second day to about the same or a little lower than that of the controls.

In the second place, it will be noticed that the curves of actual and of percentage increments in area show very marked irregularities. A discussion of the general form of the curves is therefore deferred, till after an attempt has been made to discover the causes and to reduce the magnitude of these irregularities.

The Internal and External or Daily Environmental Factors in Growth.

When the curves of daily percentage increments in area were plotted for each of the six batches of plants separately, they all showed the same general trend which seemed to be correlated with the age of the leaf, but this general trend was largely masked by considerable fluctuations which were correlated with the date. For example, there was a marked negative fluctuation on the 19/20th February in all six curves, though the period since the expansion of the leaves and the operation was different in each series on that date. It will be useful to analyse the causes of these fluctuations with a view to devising a method by means of which they may be at least partially eliminated. The percentage rate of growth in area may, *a priori*, be expected to be influenced by a number of different factors, some of an internal nature correlated with the age and stage in development of the organ or plant, others of an external nature, like light and temperature, which may be expected to vary with the hour and the date. F. F. Blackman (1) has pointed out that 'When a process is conditioned as to its rapidity by a number of separate factors, the rate of the process is limited by the pace of the "slowest" factor'. This fundamental concept introduces valuable simplicity into the study of the influence of many factors upon the rapidity of various processes; for within the range of intensities during which a factor is limiting such a process, the rate of this process will be dependent upon one variable alone—the intensity of the limiting factor—and the relation of the rapidity of the process to the intensity of the factor can therefore be calculated. In the present instance, however, the problem is more complicated. The ideal expression of the rate of growth would be in terms of the amount of actual growing substance present. As this is unknown it has been necessary to express the rate of growth in terms of a known quantity—the units of area. If the amount of growing substance per unit area were known to remain constant no difficulty would be introduced, but such a simple relation between growing substance and leaf area is, on the face of it, very improbable. The observed rate of increase in area will be, therefore, the resultant of two independent variables—the rate at which the ratio of growing substance to area varies, and the rate at which the area is being increased per unit growing substance; until more is known about these constituent processes, it will be impossible to apportion between them a change in the rate of growth. Both the relative amount of growing substance and the rate of increase in area per unit of that substance may be expected to be conditioned by a number of separate factors, some internal and correlated with the age of the organ or plant, and some external and correlated with the date. The most that can be attempted at the present state of knowledge is to make some sort of separation between the relative effects of these two groups of factors. Both the internal factors and the external daily factors are important subjects for study, but, as they are independent variables, to study one it is necessary to know to what extent the observed change in the rate of growth from one day to the next is due to the other. This has long been recognized, and the difficulty has usually been met in one of two ways; these will be described in the next section. As neither method is applicable to the interpretation of the present data, a third method will have to be employed. This latter type of method does not seem to have been suggested before—at least, I have been unable to find a reference to such a method in the literature. It will, therefore, be described in full.

Former Methods of estimating the Relative Influences of Internal and External or Daily Factors upon the Rate of Growth.

The relative influences of the internal factor and the daily factor upon growth are usually estimated in one of two ways: either the external conditions are kept as constant as possible, or else a record is kept of their fluctuations by means of self-recording instruments. The principle underlying the first method consists in keeping one of the two variables constant in order that the observed daily changes in the rate of growth may be due to the influence of the other alone. It will be convenient to call such a curve of growth, in which the fluctuations due to the daily factor have been eliminated, an 'ideal' curve of growth; a curve of this type should reveal the progressive changes in the organ's potentiality for growth, during the successive phases of its development. Having obtained such an ideal curve, it is possible to get some insight into the effect of different intensities of the various external factors upon the rate of growth at different stages in development, by varying the intensity of each factor in turn whilst maintaining the other conditions constant. The deviation of the value of the observed rate of growth from the corresponding value in the ideal rate of growth already obtained should be a measure of the effect of the change in the external agency. This method is very valuable and has been much used by plant physiologists; it has, however, some serious limitations. In the first place, it is very difficult in practice to keep external conditions constant; in many cases it is quite impossible to do so, as, for instance, in the case where the experiment has to be done on a large scale in a field. Moreover, even the form of the ideal curve may depend upon the relative intensities at which certain external conditions are kept constant; for instance, if the relative intensities of light and temperature are such that light is limiting the rate of growth, the form of the ideal curve may be different from what it would be if temperature were limiting growth. This point is strikingly demonstrated by Gregory (8) in some of his experiments on *Cucumis*; the form of the curve of growth he obtained, when light was the limiting factor, was quite different to that which he obtained when light was not limiting. Not only the form, but the steepness of the ideal curve may be different in different cases; for example, at a relatively high temperature the successive stages in development, and their correlated rates of growth, may be passed through in a shorter period of time than at a lower temperature. The form of the curve will depend upon which external agency is limiting the rate of growth, its steepness upon the intensity of this limiting factor; in presenting a curve as an ideal curve of growth, therefore, it is always necessary to specify the special conditions under which it was obtained. The most important ideal curve of growth is that which would occur in nature if the influence of the daily factor remained constant at its mean for the whole period of the experiment. As the mean daily factor will vary with the season, it is obvious that this natural ideal curve must do so too.

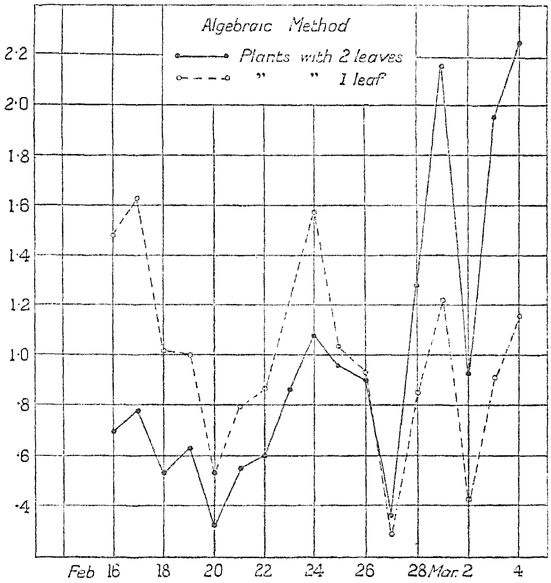
The second method, that of growing plants under natural conditions and keeping a record of the fluctuations in environmental conditions, has been employed with success by Briggs, Kidd, and West in some unpublished work on growth in *Helianthus*. This method, too, has its limitations; as the observed rate of growth is the outcome of the combined influence of two variables, the internal and the daily factors, it is necessary to know the magnitude of the influence of the one in order to estimate that of the other. If the form of the ideal curve for the mean daily factor is known, it will be possible to correlate the fluctuations from the daily expected values of the rate of growth with recorded intensities of various external agencies. But if it is the form of this ideal curve that is sought, then some previous knowledge must be possessed as to the magnitude of the deviations to be expected at each of the recorded intensities of the external agencies. It is impossible to obtain an insight into the influence of both factors at the same time from the same set of data by the use of this method.

In the case of the present experiment neither method is applicable; for external conditions were not maintained constant, and no records were kept of their daily fluctuations. Owing to the fact, however, that there were three identical series of plants starting on successive days, there seemed to be some hope that it might be possible to devise a method, based upon a new principle, by means of which the daily fluctuations due to the daily factor might be estimated and eliminated, so as to reveal the ideal curve of growth corresponding to the mean daily factor of the period of the experiment. Three such methods were worked out; they will be described in detail in the Appendix. A brief explanation of the principle upon which they were based, a sketch of their nature, and some remarks on their relative reliability will form the subject of the next section.

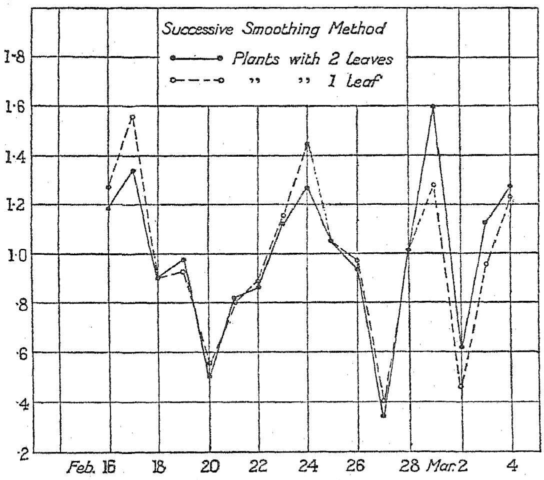
New Method of estimating and eliminating Influence of Daily Factor.

It will simplify the explanation of the principle underlying the methods now to be described if certain words are used in a rather special sense and certain symbols adopted. The twenty-four hour periods between the making of successive prints from the same leaf were not exact calendar dates, but were portions of two such dates; for the sake of brevity each of these periods will be called by the date upon which it ended. For example, in Series I the first prints were made between 10 a.m. and noon on the 15th February, and the second prints between the same hours on the 16th; this period will be referred to as that of the 16th. In the second place, the successive periods of twenty-four hours after the operation will be referred to as 'days of the experiment'; as the operation was performed on a different day in each series, these successive days of the experiment will likewise correspond to different dates in the three cases. Thus, using the dates as defined above, the first 'day of the experiment' will correspond with the dates 16th, 17th, and 18th February in Series I, II, and III respectively. It has been pointed out that the magnitude of each observed rate of growth will be the outcome of the influences of two independent variables, the internal factor and the daily factor, and by definition the internal factor will be correlated with the age of the organ, and hence with the 'day of the experiment', whilst the daily factor will be correlated with the date. The successive values of the growth magnitude that would have been obtained had the daily factor remained constant at its mean for the whole period of the experiment have been termed the 'ideal values'; the ideal values for the successive days of the experiment will clearly be the outcome of the influence of one variable, the internal factor (correlated with the 'day'), and a constant, the mean daily factor. It will be convenient to denote the values of the daily factor on the seventeen successive dates of the experiment (16th February to 4th March) by the symbols $p_1, p_2, p_3, \ldots p_{17}$, and to denote the mean of these seventeen values of the daily factor by the symbol P. In the same way the 'ideal' values of the rates of growth on the successive 'days of the experiment' will be denoted by the symbols $y_1, y_2, y_3, \ldots$ By definition these 'ideal' values are those that would have been obtained had the daily factor remained constant at the value P. The observed rate of growth on each 'day' will be the function of these two variables. Thus those for the first 'day' may be written $f(y_1), f(p_1)$; $f(y_1), f(p_2)$; and $f(y_1), f(p_3)$, in the case of Series I, II, and III respectively; those for the second 'day' may be written $f(y_2), f(p_2)$; $f(y_2), f(p_3)$; and $f(y_2), f(p_4)$. In comparing the three observed values for the first 'day', the internal factor becomes a constant, i. e. it is y_1; the differences between the three values will therefore be due to the differences in the values of the daily factor (p) alone; it should be possible therefore to calculate the relative value of p_1, p_2, and p_3. Similarly, from the three observed values for the second 'day' it should be possible to find the relative magnitudes of p_2, p_3, and p_4, and from the three values for the third 'day' the relative magnitudes of p_3, p_4, and p_5, and so on for all values of 'p'. In order to discover the actual values of p_1, p_2, &c., that is to say, the actual deviations of the daily factors from their mean 'P', it will be necessary to know the value of 'P', and to know which of the two following possibilities holds true. The observed value of the growth magnitude may be the sum of the two variables 'y' and 'p', or it may be their product. In the first case the observed values for the first 'day' will be y_1+p_1, y_1+p_2, and y_1+p_3; in the second case they will be y_1p_1, y_1p_2, and y_1p_3. If the first of these two possibilities holds true, if, that is to say, the influence of the daily factor is 'additive' and independent of the value of y, then by definition $y_1+P=y_1$ and, hence, $P=0$; if, on the other hand, the second possibility holds true, then by definition $y_1P=y_1$ and $P=1$. As there is no *a priori* reason for deciding which of the two possibilities is true, three methods were worked out, two based on the assumption that the observed value of the rate of growth may be written yp, and one on the assumption that it may be written $y+p$. It is obvious that in the latter case the daily factor is the number that must be subtracted from or added to the observed rate of growth in order to obtain the 'ideal' rate of growth, whilst in the former case it is the number by which the observed rate must be divided; i.e. in the one case $y+p-p=y$, in the other case $y \times p/p=y$. A full description of the methods will be given in the Appendix, and only brief mention of them made here. Instead of calculating the successive values of the daily factor from all the plants in each series, advantage was taken of the fact that there were two batches of plants in each series, one with two leaves and the other with one, and the daily factors were worked out independently from these two sets of data. Theoretically, the two values thus found for the daily factor on each date should be identical, and a measure of the accuracy of the method was thus provided by the degree to which these two values approximated to one another. When 'correcting' the observed values of the rate of growth on a date by the daily factor for that date, in order to obtain the 'ideal' value for the rate of growth, the mean between these two values of the daily factor was used, as it is likely to be more correct than either taken singly, being based on a larger number of observations. The observed values for the rate of growth on each date were 'corrected' by the daily factor for that date in each of the six batches of plants separately, and the mean 'ideal' values of the rate of growth on each 'day of the experiment' worked out from the three batches of plants with one leaf, and from the three batches of plants with two leaves.

The additive method, i.e. that based upon the assumption that the observed rates of growth may be written $y+p$, gave very unconvincing results; not only were the two values for the daily factor on each date divergent, but the 'ideal' curves of growth obtained by applying the mean daily factors to the 'correction' of the observed rates were more irregular and less convincing than the original uncorrected curves; this method was therefore abandoned. Of the two methods based on the assumption that the observed rates of growth may be written yp, one is algebraic in nature and is founded upon certain suggestions made to me by Mr. Udny Yule, the other consists in a form of successive smoothing. The two values found for '*p*' on each date from the plants with one leaf and those with two respectively by the algebraic method are plotted on Graph B; it will be



GRAPH B. Comparison of the two values of the daily factor for each date found from plants with one leaf and with two leaves respectively by the algebraic method.



GRAPH C. Comparison of the two values of the daily factor for each date found from plants with one leaf and with two leaves respectively by the successive smoothing method.

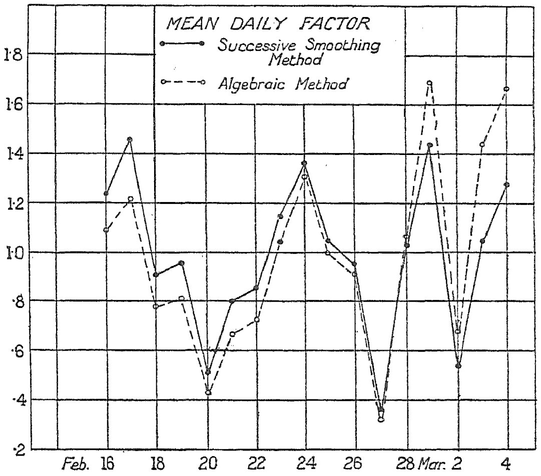
observed that they are widely divergent. The corresponding pairs of values of '*p*' found by the successive smoothing method are given in Table III, together with the mean values, and are plotted on Graph C; on the whole, the two values on each date agree very well. The mean values of the daily factor (p) are plotted on Graph D, where they are compared with the corresponding mean values found by the algebraic method; it is of interest to note that there is again a fair general agreement. In order to find out how many times in succession the smoothing process must be applied in order to obtain the 'ideal' values with reasonable accuracy, a logarithmic curve was distorted by means of a number of arbitrary errors in a manner similar to that assumed to have taken place in the 'ideal' curve through the agency of the daily factor, and the distorted curve was 'corrected' by the method; after the fourth successive smoothing it was found to have regained approximately its original form; four repetitions of the smoothing process were, therefore, considered sufficient when correcting the original experimental data.

TABLE III.

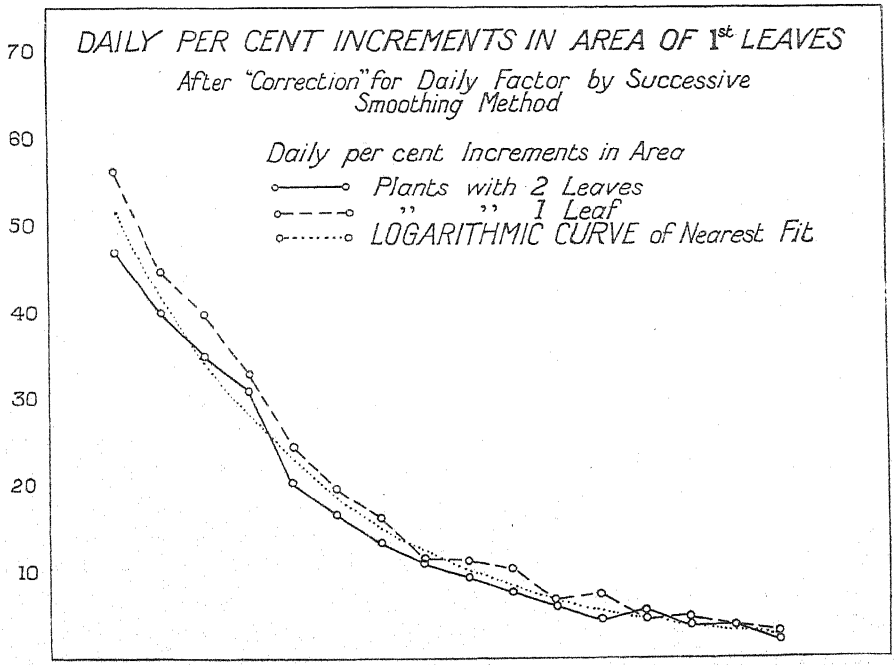
Values of Daily Factor and of 'Ideal' Rates of Growth found by the Successive Smoothing Method.

Date on which 24-hour Period ended.	*Daily Factor.*			*Day of Experiment.*	*Mean Curves of Growth after 'Correction' by Mean Daily Factor.*	
	From Plants with 2 Leaves.	*From Plants with 1 Leaf.*	*Mean.*		*Plants with 2 Leaves.*	*Plants with 1 Leaf.*
Feb.						
16	1·1910	1·2930	1·2420	1	46·89	56·33
17	1·3440	1·5730	1·4585	2	40·07	44·69
18	0·8980	0·9099	0·90345	3	34·99	40·07
19	0·9822	0·9383	0·96025	4	31·07	33·02
20	0·5060	0·5504	0·5282	5	20·38	24·45
21	0·8123	0·8097	0·8110	6	16·79	19·65
22	0·8480	0·8616	0·8548	7	13·37	16·29
23	1·1330	1·1600	1·1465	8	11·22	11·49
24	1·2710	1·4620	1·3665	9	9·36	11·32
25	1·0420	1·0420	1·0420	10	7·64	10·35
26	0·9419	0·9740	0·95795	11	6·39	6·86
27	0·3370	0·3942	0·3656	12	4·54	7·35
28	1·0400	1·0350	1·0375	13	5·40	4·66
March						
1	1·6030	1·2980	1·4505	14	3·88	4·78
2	0·6200	0·4620	0·5410	15	3·70	3·76
3	1·1300	1·9779	1·05395	16	2·02	2·98
4	1·2940	1·2570	1·2755	—	—	—

In addition to the 'ideal' values of the daily percentage increments in area which are given in Table III and are plotted on Graph E, the successive 'ideal' values of the 'actual areas' were calculated. This was done as follows. If A_1 is the area of the leaf at the beginning of the first day of the experiment and y_1 the percentage increment in area during that period, clearly



GRAPH D. Comparison of the two mean values of the daily factor found for each date by the algebraic and the successive smoothing methods respectively.



GRAPH E.

$\left(A_1 + A_1 \frac{y_1}{100}\right) = A_2$, or the area at the end of the first twenty-four hours, that is to say, $A_2 = A_1\left(1 + \frac{y_1}{100}\right)$.

Similarly,

$$A_3 = A_2\left(1 + \frac{y_2}{100}\right) = A_1\left(1 + \frac{y_1}{100}\right)\left(1 + \frac{y_2}{100}\right).$$

As the initial mean areas of the leaves in the plants with one leaf and the control plants were slightly different, being 5·56 and 5·83 sq. cm. respectively, the mean for all the leaves, 5·65 sq. cm., was taken as the initial value and the successive 'ideal' areas calculated. For example, at the end of the first day the 'ideal' value in the case of the plants with one leaf was $5{\cdot}65\left(1 + \frac{46{\cdot}89}{100}\right)$, that is, $5{\cdot}65 \times 1{\cdot}4689$ or 8·301 sq. cm.; whilst in the case of the control plants it was $5{\cdot}65\left(1 + \frac{56{\cdot}33}{100}\right)$ or 8·831 sq. cm. These 'ideal' areas will not be tabulated, but the 'ideal' actual increments found from them are plotted on Graph F, where they are compared with the corresponding original values in the uncorrected data.

DISCUSSION OF EXPERIMENT I.

Magnitude of the Fluctuations due to the Daily Factor.

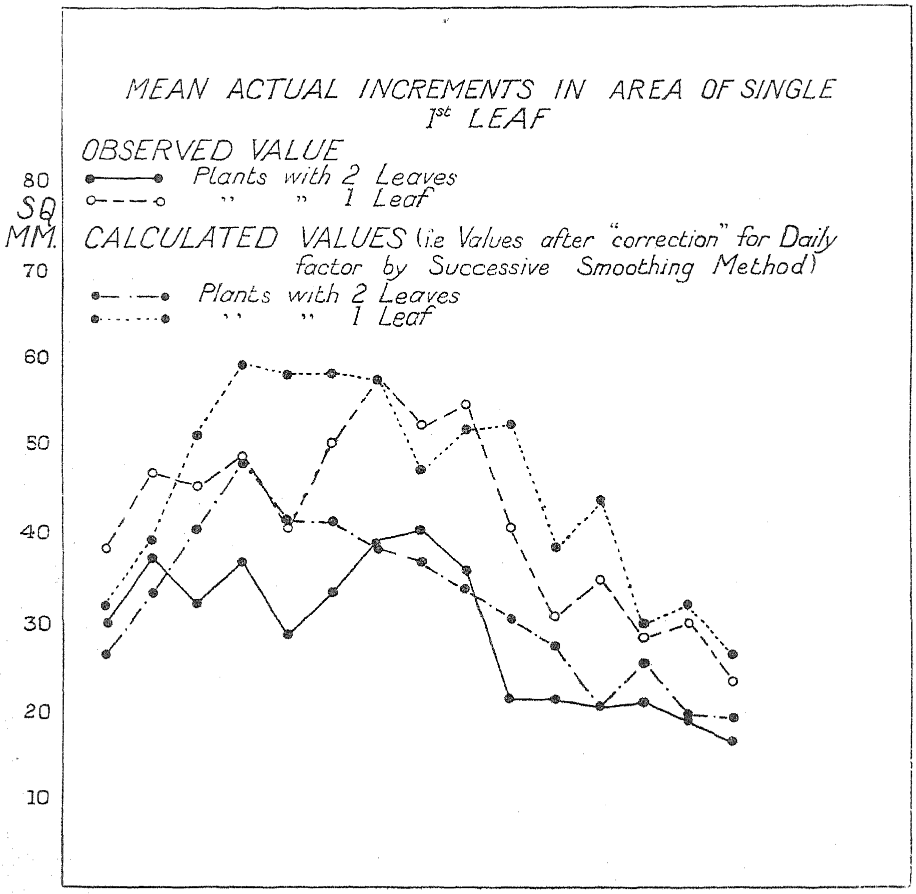
The successive values of the daily factor, found by the successive smoothing method, have been given in Table III and in Graph E. It is important to remember that these values do not represent the absolute magnitudes of the daily influences of external agencies upon the rate of growth; they merely represent the daily deviations of the daily factor from a constant, its mean value for the period of the experiment. Similarly, the ideal values for the rate of growth, given in Table III and Graph E, are those specific for this particular mean value of the daily factor; they represent the quotient of the 'fundamental' ideal values divided by this mean daily factor, where the 'fundamental' ideal values are those that would have been obtained had no external agency been limiting the rate of growth either directly or indirectly. If the mean daily factor had been different, the ideal values would have been different also. It would only be possible to define an ideal curve as 'fundamental' and determined solely by internal factors correlated with the age of the organ, if it were known that the intensities of all the external agencies were such that none of them were limiting the rate of growth either directly or indirectly; the values of the growth magnitudes in such a case would be determined entirely by some internal limiting factor. It is obvious that under such circumstances the observed growth magnitudes would show no fluctuations correlated with the date; they would themselves constitute the fundamental ideal curve. The fact that there are marked fluctuations in the case of the data under consideration, and that these fluctuations are correlated with the date, is strong evidence that some external agency (or agencies) was limiting the rate of growth. As the fundamental ideal curve is not known in this instance, the 'absolute' value of the daily factor is unknown also; furthermore, the daily deviations of the daily factor from the mean daily factor cannot be correlated with daily fluctuations in the intensity of any particular external agency, as no record was kept of the fluctuations of these agencies; the method could be applied to such a purpose, however, if such records were kept. All that can be extracted from the calculated values of the daily factor is some idea as to the magnitudes to be expected in the daily deviations of the daily factor from the mean daily factor during a period of seventeen days. These are very considerable indeed, and show that little reliance can, with safety, be placed in the significance of a series of growth magnitudes on successive dates, unless the relative values of the daily factor on those dates can be calculated and allowed for. The values of the daily factor in the present instance, i. e. the numbers by which the observed growth magnitudes must be divided to obtain the ideal values specific for the mean daily factor, range from 1·4585 (on 17th February) to 0·3656 (on 27th February).

Form of the Curves.

The curves of mean actual areas on successive days have already been given in Graph A; both in the case of the plants with one leaf and in that of those with two the curves are approximately of the well-known S form. Robertson (9), commenting upon the prevalence of this type of curve, suggests that 'any particular cycle of growth obeys the formula $\log. \frac{x}{A-x} = k(t-t_1)$, where x is the amount (in weight or volume) of growth that has been attained at time t, A is the total amount of growth attained during the cycle, k is a constant, and t_1 is the time at which growth is half completed', and points out that 'the above relations are such as would be expected to hold good were growth the expression of an autocatalytic chemical reaction'. Gregory (8) has shown that 'the curves of increase in area and in linear dimensions for a single leaf' (of *Cucumis sativus*) 'in daylight are of S form, and can be fairly represented by the formula of an autocatalytic reaction in which the material catalysed gradually decreases in amount as the reaction proceeds'.

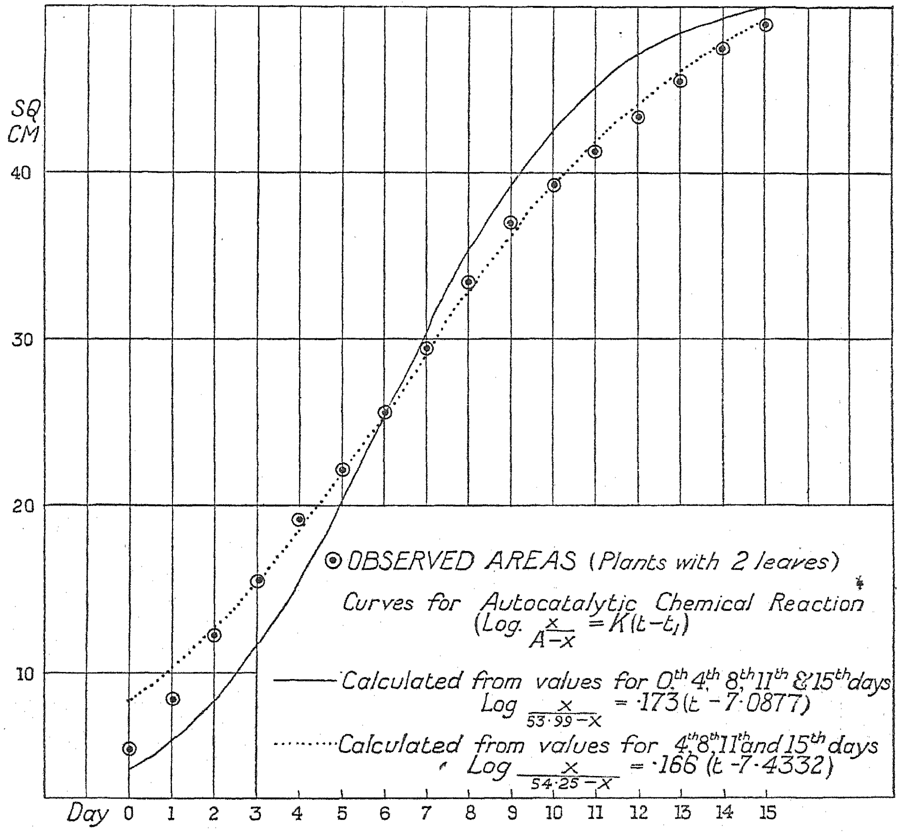
In Table IV the observed mean areas of the leaf on successive days are compared with 'ideal' values calculated by means of Robertson's formula. Four columns of calculated ideal values are given, based on values of A, k, and

t_1 derived as average values from the different trial selection of four days set out at the heads of the respective columns. The calculated values of the first column fit the corresponding observed values very badly, those of the other columns fit fairly well, except on the earlier days of the experiment. On Graph G the values in the first and third columns are plotted and compared with the curve of observed daily areas in the case of plants



GRAPH F.

with both leaves left intact. It is of interest to note that in Gregory's data (8, Table IV, p. 100) there is a similar discrepancy between the earlier portions of the observed and calculated curves of growth. This discrepancy would be accounted for if it could be shown that there are two independent and overlapping cycles of growth involved, and, as will be pointed out later, there is evidence for this in the case of the data of the present experiment; but, as West, Kidd, and Briggs point out, it seems doubtful if there is any fundamental significance in the resemblance. These authors (10) 'agree with Enriques' (7), 'that the similarity between an autocatalytic reaction and growth is a purely superficial one, the falling off in rate per unit dry weight being due rather to increasing differentiation into productive and non-productive tissues'. Curves of nearest fit based on Robertson's formula were also worked out from the smoothed 'ideal' values of the daily areas; as the same general relations were found to hold in that case also, the details need not be entered into further.



GRAPH G.

The curves of observed actual increments in area and the corresponding curves of 'ideal' or smoothed increments have been given on Graph F; the former show marked fluctuations which have not been completely eliminated in the latter. Both sets of curves, however, seem to belong to the same general type, that known as Sachs's Grand Period curve of growth; there is an initial rise to a maximum followed by a decline; the position of this maximum, however, is earlier in the smoothed than in the observed curves. The magnitude of the increment in area of a leaf during each period will presumably be dependent upon two factors—the amount of growing substance or 'productive capital' present, and the rate at which the leaf increases in area per unit of this substance; these two factors may be, and probably are, independent variables. The rise of the curves to a maximum during the first few days of the experiment, i. e. after the expansion of the first leaves, indicates that either the amount of growing substance or the rate of increase in area per unit of this substance, or both, at first increases; the decline in the curves during the later days indicates that one or both of these factors subsequently decreases. The data does not permit further analysis.

TABLE IV.

Observed Areas of Leaves compared with Values calculated from the Formula for an Autocatalytic Reaction. Log. $\frac{x}{A-x} = k(t-t_1)$.

	Plants with Both Leaves left intact.					Plants with One Leaf cut off.				
		Values calculated from Areas on—					Values calculated from Areas on—			
Day of Expt.	Observed Areas.	Days 0, 4, 11, and 15.	Days 2, 6, 11, and 15.	Days 4, 8, 11, and 15.	Days 6, 10, 11, and 15.	Observed Areas.	Days 0, 4, 11, and 15.	Days 2, 6, 11, and 15.	Days 4, 8, 11, and 15.	Days 6, 10, 11, and 15.
0	5·56	4·19	7·76	8·25	8·23	5·83	4·01	8·72	9·64	9·42
1	8·54	5·98	9·81	10·30	10·25	9·68	6·01	11·29	12·24	12·03
2	12·27	8·45	12·28	12·72	12·73	14·37	8·88	14·48	15·43	15·20
3	15·50	11·66	15·11	15·50	15·47	18·93	12·84	18·28	19·16	18·95
4	19·175	15·63	18·33	18·62	18·56	23·80	18·08	22·71	23·44	23·28
5	22·06	20·26	21·85	22·00	22·00	27·88	25·12	27·67	28·19	28·08
6*	25·4	25·43	25·55	25·57	25·57	32·93	31·93	33·07	33·23	33·26
7	29·39	30·38	29·32	29·18	29·20	38·67	39·67	38·63	38·60	38·49
8	33·435	35·10	32·99	32·73	32·74	43·93	46·54	44·17	42·62	43·91
9	37·04	39·21	36·45	36·06	36·13	49·43	53·42	49·39	48·87	48·94
10	39·18	42·48	39·60	39·15	39·22	53·50	58·62	54·16	53·47	53·58
11	41·325	45·12	42·37	41·89	41·98	56·57	62·53	58·32	57·59	57·69
12	43·39	47·06	44·72	44·25	44·36	60·05	65·73	61·84	61·15	61·20
13	45·51	48·43	46·67	46·25	46·46	62·90	67·33	64·76	64·13	64·16
14	47·42	49·39	48·31	47·89	48·05	65·87	68·85	67·11	66·59	66·58
15	49·06	50·06	49·60	49·25	49·47	68·20	69·58	68·96	68·58	68·47
	A	53·99	53·99	54·25	54·45	A	71·29	74·92	75·51	75·08
	k	0·173	0·121	0·116	0·116	k	0·189	0·1296	0·122	0·124
	t_1	7·0877	7·4007	7·4332	7·4574	t_1	7·4799	7·7893	7·8405	7·8032

The curves of observed percentage increments in area have been given on Graph A, the corresponding curves corrected for the daily factor by the successive smoothing method on Graph E, where they are compared with the logarithmic curve of nearest fit. Both sets of curves start at their highest point and descend in a somewhat logarithmic manner, but in the curves of observed values there are considerable fluctuations and an indication of a secondary maximum on about the sixth day after the expansion of the leaves. In the smoothed curves these fluctuations have been reduced and the secondary maximum has vanished; the latter was probably due, therefore, to a chance occurrence of days especially favourable for growth. As it will be shown that these curves of percentage increments in area are really of a compound nature, at any rate in their earlier portions, their apparently logarithmic form is probably not of much significance, except perhaps in their later portions.

Effect of removing One of the Two First Leaves the Day they expand.

It has already been pointed out that, where one first leaf was cut off the day those organs expanded, the remaining leaf grew during the next fifteen days to a mean area about 40 per cent. larger than that attained by a single leaf where both leaves were left intact. Compensating growth took place, in other words, but not sufficient to make up for the loss of the leaf removed.

The actual increments in area were consistently higher in the case of plants with one leaf removed than in those with both left intact; as the initial amount of growing substance present at the time when the operation was performed was presumably the same in each case, this higher rate of growth must have been due on the first day to the rate of increase in area per unit growing substance being greater in the case of plants with only one leaf. The higher rate on the later days may have been due to the same cause, or it may on the other hand have been due to there being a larger amount of growing substance present after the first day's growth, or both causes may have co-operated to bring about the result.

The curves of percentage increments in area, corrected for the daily factor, have been given on Graph F; for over a week the curve for plants with one leaf is consistently higher than that for those with two leaves, and, on the whole, it is a little higher during the later days of the experiment too. This higher rate of increase in area may have been due to the presence of a larger amount of growing substance, or to a higher rate of increase in area per unit growing substance, or to both; on the first day of the experiment it was presumably due to the latter of the two factors.

INTERPRETATION OF RESULTS OF EXPERIMENT I.

The rate of increase in area was relatively more rapid when one leaf was removed than when both were left intact; it remains to formulate some hypothesis that will account for this compensative growth. It seems clear that the rate of increase in area, where both leaves were left intact, must have been limited by some factor whose intensity was altered by the removal of one leaf. To have conditioned compensative growth, therefore, a factor must fulfil two qualifications: it must be of a character likely to limit the rate of growth and liable to be altered in intensity by the removal of one leaf. There are two alternative agencies that seem to fulfil these qualifications: one is the water relationship, the other the supply of food. As regards the former, the water relationship depends on the balance between the amount of moisture absorbed by the roots and the amount lost through transpiration by the shoot; as the leaves are the main transpiring organs of the plant, removal of one, when there are only two, will reduce the transpiring surface by something approaching one-half, whilst the water-absorbing system will remain unchanged. The remaining leaf will be more favourably placed as regards its water-supply than the two leaves where both are left intact. In the latter case relative lack of water might occasion partial closure of the stomata, with a consequent reduction in gaseous exchange and in the rate of photosynthesis; a lower turgor, moreover, might occasion a slower extension of the cells. The claims of the water relationship, therefore, must not be neglected when considering the conditions correlated with compensative growth. The alternative agency mentioned above was the supply of available food. If the leaves were dependent entirely upon their own photosynthetic activity for the food required for growth, it would be hard to discover a reason why the removal of one of these organs should increase the supply at the disposal of the survivor; in fact, the reverse might be expected to occur. For the removal of the leaf would deprive the plant of but one of many centres of potential growth, whilst its loss would halve the photosynthetic sources of supply. The reduction in the supply would be relatively greater than the reduction in the demand for food; hence the rate of translocation of food material from the leaf would probably be increased, with a resulting reduction in the concentration of those substances within that organ. But it seems certain that the leaf, when young, has other sources for its food material besides that produced by its own photosynthetic activity; during the period before it expands this must obviously be the case. The food withdrawn by the leaf from the general fund of the plant must be greater at this period than that contributed by the leaf to that fund. It is uncertain for how long after the expansion of the leaves this state of affairs is maintained, but it is clear that the removal of a leaf at this stage in development would reduce the consumption of food more than it reduced the immediate food supply; the remaining leaf would be placed in more favourable circumstances as regards its supply of food material, and might have its rate of growth increased—provided, of course, the amount of this material was the factor limiting the rapidity of that process.

From the isolated data of the present experiment it would not be possible to decide which of these alternative hypotheses contains the greater inherent probability.

The experiment to be described next provides strong evidence that, for several days after they expand, the leaves derive much of their food from the reserves stored in the cotyledons. This points to the second of the alternative hypotheses being the more probable. The most rational interpretation of Experiment I may therefore be stated as follows:

For several days after the expansion of the first leaves the supply of available food is limiting the rapidity of their increase in area; a considerable portion of this supply of food is derived from the reserves stored in the cotyledons. When, therefore, one of these leaves is removed the day it expands, the remaining leaf has at its disposal a supply of food from this source which, when both leaves are left intact, has to be divided between the two; the rate of growth of this surviving leaf is consequently more rapid than that of a leaf where both are left intact.

EXPERIMENT II.

Effect of removal of cotyledons upon rate of growth of first leaves.

Object. Experiment I showed that considerable compensative growth takes place in one first leaf if the other is removed the day those organs expand. This implies that the rate of growth of those organs is limited by some factor, and that the intensity of this factor is altered by the removal of one leaf. It has been pointed out that there are two alternative agencies, the water relationship and the supply of available food, either of which might be limiting growth and altered in intensity by the removal of one leaf. The aim of Experiment II was to explore further the relative probabilities of these alternative hypotheses. For this purpose it was necessary to devise a method of treatment that would alter the intensity of one of the factors and leave that of the other unchanged. The removal of the cotyledons the day the first leaves expanded seemed to be a mode of treatment that fulfilled these requirements; it is hard to see how the removal of those organs could greatly alter the water relationship in the plant, and in so far as it altered it at all, it might be expected to decrease transpiration rather than to increase it; it is easy, on the other hand, to see how the removal of those organs with their reserves of food might decrease the amount of food available for growth. If, therefore, a decrease in the rate of growth of the leaf resulted from the removal of the cotyledons, the inference would be that the leaf is deriving much of the food it requires for growth from the reserves stored in those organs, and of the two alternative interpretations of the results of Experiment I that postulating a change in the amount of available food would be the more probable. If, however, no change in the rate of growth resulted, then the compensative growth was probably due to a change in the water relationship rather than to a change in the amount of available food.

Treatment of plants. The apical bud, one first leaf, and both cotyledons were removed from a number of plants the day their first leaves expanded, whilst from control plants of the same age and the same stage of development the apical bud and one first leaf were removed, but the cotyledons left intact. To show the influence of the removal of the cotyledons at a slightly later stage of development, these organs were removed from some of these control plants two days after the expansion of the first leaves. From another batch of plants of the same age, whose leaves expanded a day later, the apical bud and one first leaf were removed on that day, and the cotyledons removed from some of these plants six days later.

History. Eighty seeds of between 0·7 and 0·8 grm. in weight were sown in tin tubes, as before, on 21st April, and a number of young plants were transplanted into pots when they showed above ground. The first leaves of one plant expanded on the 29th April, those of another eighteen plants on the 30th. On the latter date the apical bud and one first leaf were removed from each of these nineteen plants and the cotyledons from six of them; two days later the cotyledons were cut off another six of these plants. Prints were made from the remaining leaf of each plant every day for a week. The first leaves of another fourteen plants expanded on 1st May, and these plants were constituted into a second series. The apical bud and one first leaf were removed on that date, but the cotyledons were at first left intact; six days later the cotyledons were removed from five of these plants. Prints from every leaf were made each day as before, the last prints being made the day after the removal of the cotyledons.

Again there were some casualties,[1] and the number of plants that survived in the case of the respective batches was as follows: Series I, plants from which the cotyledons were removed the day the first leaves expanded, six plants; those from which the cotyledons were removed two days later, five plants; those whose cotyledons were left intact the whole period, five plants; Series II, plants whose cotyledons were removed on the 7th May, three plants; those whose cotyledons were not removed, nine plants.

RESULTS OF EXPERIMENT II.

In presenting the results of this experiment only the mean percentage increments will be given; these will be found in Table V and are plotted on Graphs H and J. As there were no identical series started on successive days, it is not possible in the case of the present data to estimate the magnitudes of the fluctuations due to the daily factor, or to calculate the ideal curves of growth specific for the mean daily factor. This, however, is of little importance in the present instance; whether the cotyledons were removed on the same day as the first leaf and the apical bud, or on the second or the sixth day after the removal of those organs, the mean rate of growth of the surviving leaf on each date is compared with that which occurred on the same date in the case of control plants of the same age and in the same stage of development whose cotyledons were left intact.

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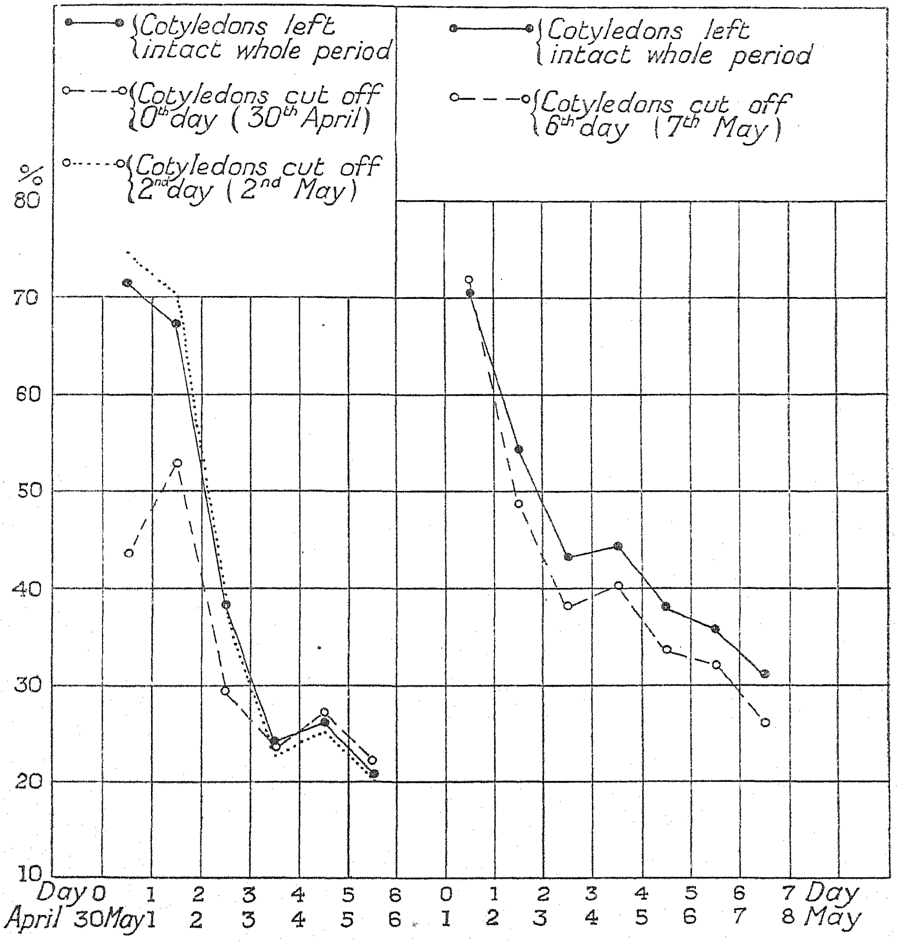
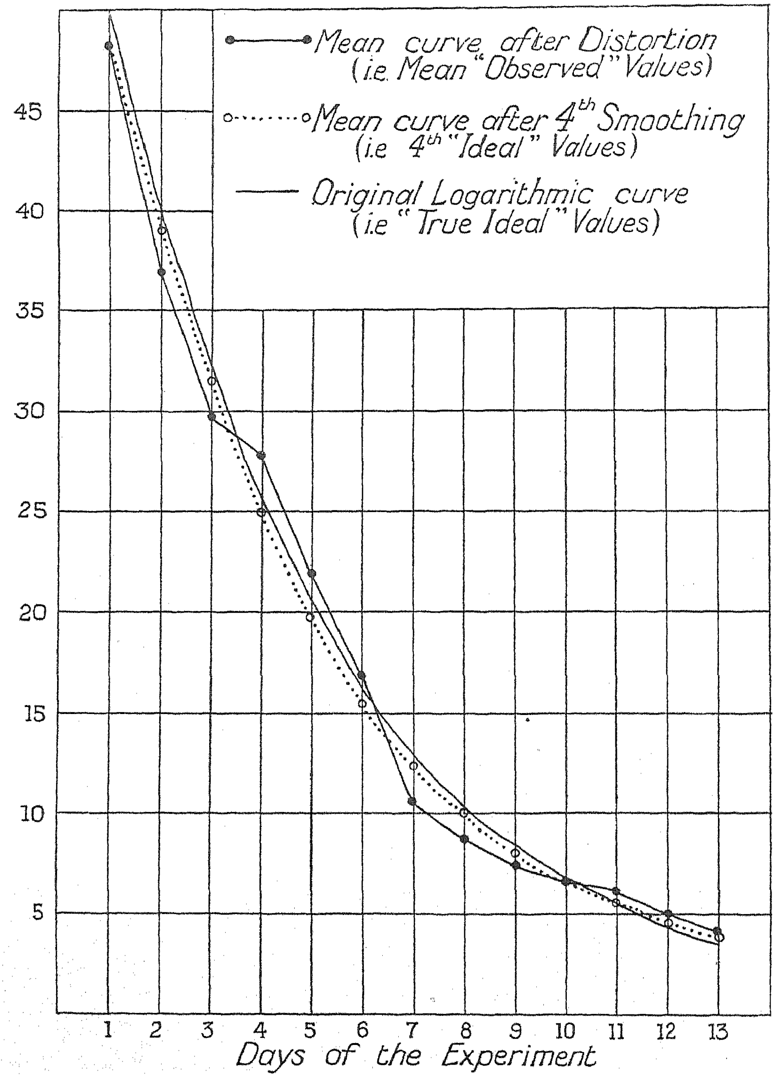
[1] The plant whose leaves expanded on the 29th April—the day before the operation—grew slower than those similarly treated whose leaves expanded on the 30th, and another plant in Series I had only a very small damaged leaf which at first grew much slower than those of the other plants; as these plants were not strictly comparable to the rest, the figures for their growth were excluded when working out the mean rates of growth. One of the plants whose cotyledons were removed on 2nd May became damaged on that day and died on the 3rd, whilst the prints taken from the leaves of two plants whose cotyledons were removed on the 7th May stuck to the ferrotype plate and could not be used.



GRAPH H.

Hence in the case of each individual comparison between a pair of observed growth magnitudes, the periods of time concerned were contemporary and identical—the daily factor must have been the same in the case of each member of the pair. Where the rates of growth were different in the two members of such a pair of observations, this difference cannot have been due to a difference in the intensity of some external factor; where the rates of growth were the same, however, in both members of the pair, there is a possibility which must not be overlooked that this value of the growth magnitude may have been due to the rate of growth being limited at that level in each case by the intensity of some external agency.



GRAPH J.

If Table V and Graphs H and J are examined, it will be noticed that removal of the cotyledons on the day the first leaves expanded resulted in a marked decrease in the rate at which the leaf increased in area. The growth magnitude on the first day was only about 40 per cent., whilst that in the case of the controls with cotyledons intact was as much as 71 per cent. The growth magnitudes for the second period of twenty-four hours were 52 and 67 per cent. respectively in the case of the plants without cotyledons and those with cotyledons; the corresponding values for the rate of growth during the third period of twenty-four hours were 29 and 38 per cent. respectively; during the fourth and subsequent periods of twenty-four hours the rates of growth were approximately the same in both batches of plants. When the cotyledons were removed on the second day after the expansion of the leaves, there was no appreciable change in the relative rate of growth. The result of removing the cotyledons on the sixth day after the expansion of the leaves was unfortunately obscured by the fact that the mean rate of growth of the small number of plants so treated happened to be already considerably lower than that of the corresponding controls, but there seems to have been no very appreciable further drop in the rate of growth.

Table V.

Daily percentage Increments in Area of Surviving First Leaf.

	Series I.				*Series II.*		
	Apical Bud and One First Leaf cut off on 30th April.				*Apical Bud and One First Leaf cut off on 1st May.*		
Date.	*Day of Expt.*	*Cots left on Whole Period.*	*Cots cut off 30th April.*	*Cots cut off 2nd May.*	*Day of Expt.*	*Cots left on Whole Period.*	*Cots cut off 7th May.*
May							
1	1	71·40	40·35	74·50	—	—	—
2	2	67·30	52·83	70·00	1	70·06	72·00
3	3	38·28	29·50	37·90	2	54·33	48·70
4	4	24·20	23·90	22·90	3	43·22	38·20
5	5	26·20	27·00	25·50	4	44·22	40·80
6	6	20·75	22·00	20·40	5	38·00	33·70
7	—	—	—	—	6	35·72	32·30
8	—	—	—	—	7	31·44	26·00

Discussion of the Results of Experiment II.

Before drawing conclusions from the results of this experiment, some decision must be reached as to the degree of significance that may with safety be placed in the data, for there is the possibility that the differences in the rates of growth of the leaf in the case of the batches of plants subjected to different types of treatment may have been due to chance variations in the specific make-up of the plants concerned, rather than the result of a response to the respective treatments. In the first place, it will be noticed that the growth magnitudes on the successive 'days of the experiment' were very different in the two batches of control plants, though both batches had received the same treatment; for example, that on the sixth day after the operation was 20·75 per cent. in the case of the control plants in Series I, whilst it was as high as 35·72 in the case of the control plants of Series II. This discrepancy, however, does not militate against the significance of the data, since the differences in this case were probably due to fluctuations in the daily factor; for the 'successive days of the experiment' correspond to different calendar dates in the two series. Of more serious importance are the considerable differences in the rates of growth on each date to be noted in the two batches of plants constituting Series II, for both these batches received the same treatment until the sixth day and both were of the same age and were operated upon on the same date. Examination of these differences, however, reveals the fact that they were fairly uniform; except on the first day after the operation, the rate of growth in the case of the batch of three plants was always about seven-eighths that of the control batch of nine plants; the form of the curve of growth, moreover, was much the same in both batches. When the rate of growth in the plants whose cotyledons were removed on the day the first leaves expanded are compared with corresponding rates that took place in the controls, not only are the differences on the first three days found to be far greater than those in the case of Series II, but the actual forms of the curves are widely dissimilar during their earlier portions. The magnitude and the nature of these differences seem too marked to be due merely to chance, and it is significant that the curves are so similar in their later portions. The close consistency of the curve for plants whose cotyledons were cut off on the second day of the experiment to that of the control plants in the same series is a further argument in favour of placing considerable confidence in the significance of the results. The sources of possible error, however, are fairly considerable, and it will be safer to regard the results as more or less qualitative rather than strictly quantitative.

It has been pointed out that removal of the cotyledons would be more likely to reduce the supply of available food than the intensity of any other agency liable to limit the rate of growth. Removal of those organs resulted in a considerable reduction in the rate of growth of the leaf. It seems safe to conclude that the leaf draws a considerable portion of the food it requires for growth from the reserves stored in the cotyledons. Removal of those organs before these reserves are exhausted deprives the leaf of this source of supply, and relative lack of food material limits the rate of growth to a lower level. In the case where the cotyledons were removed the day the leaves expanded, the difference between the relative rates of growth in the plants without and those with cotyledons was greatest on the first day after the expansion of the leaves, and became progressively less until on the fourth day the two rates were about the same. This suggests that the amount of food derived by the leaf from the cotyledons likewise progressively decreases until it becomes on the fourth day negligible in amount.

A considerable amount of growth took place, however, even when the cotyledons were removed; this amounted to an increment of about 40 per cent. on the first day, one of about 53 per cent. on the second day, and one of about 30 per cent. on the third day. Part of this growth may have been at the expense of food that had already left the cotyledons before those organs were removed, but a later experiment—which will be described in another paper—has shown that this would not be likely to account for an increment of more than 10–17 per cent. on the first day or for one of more than 4 or 5 per cent. on the second and third days; the remaining portions of these increments were therefore presumably effected at the expense of food derived from the leaves' own photosynthetic activity. It is interesting to note that the rate of growth at the expense of food from this source was greater on the second day after the expansion of the leaves than on the first day; this suggests that the maximum rate of photosynthesis was not attained until the second day. Too much significance must not be attached to this observation, however, for this rise in the rate of growth is of an order that might be brought about by a chance occurrence of a day especially favourable for growth. In a later experiment—which will be described in a subsequent paper—a similar rise was observed in the case of plants similarly treated, and in this case the influence of the daily factor was at least partially eradicated by the successive smoothing method; consequently this rise in the rate of growth may be of real significance. It is interesting to remember that Briggs (2), working on this same species of plant, has shown that there is a rise in the rate of photosynthesis at about this stage in development. The subsequent fall in the rate of growth might possibly be due to a decrease in the amount of food derived from photosynthesis, but this would not be in accord with the modern knowledge of this process which points to its rate remaining relatively stable for a considerable time after it has attained its maximum; a different interpretation seems more probable. It has been pointed out that the observed growth magnitudes will be the outcome of the joint influence of two independent variables, the amount of growing substance per unit area and the rate at which the area is increased per unit growing substance. The subsequent decrease in the rate of growth may have been due to a decrease in the amount of growing substance per unit area. It might be said that the rise in the rate of growth on the second day of the experiment may have been due to a relative increase in the amount of this growing substance, but if that were the case a rise in the rate of growth on that day should have occurred in the case of the control plants also. Although the effect of removing the cotyledons on the day the leaves expanded was still considerable on the third day after expansion, removal of those organs on the second day had no appreciable effect. These two facts do not seem to be in accord; perhaps there is a lag between the movement of the reserve food from the cotyledons and its absorption in the process of growth. The food necessary for the full rate of growth on the third day may have already left the cotyledons before those organs were removed on the second day. It is not surprising that the removal of the cotyledons on the sixth day after the expansion of the leaves had no appreciable effect on the rate of growth; the reserves of food stored in those organs may be expected to have been exhausted by that day.

GENERAL CONCLUSIONS.

It will be profitable to review the results of the two experiments taken together and to show how the interpretations suggested in the two cases connect together as a consistent whole. If the rapidity of one of the processes governing the rate of increase in area—the rapidity, that is, of either the rate of increase of the amount of growing substance per unit area or of the rate of growth per unit growing substance—is limited at first by the supply of available food, and if, for several days after their expansion, the leaves derive a considerable portion of their supply of food from the reserves stored in the cotyledons, then it would necessarily follow that the removal of one first leaf at this stage would result in an increase in the rate of growth of the other—provided, of course, that the cotyledons were left intact. For the surviving leaf would have at its disposal a supply of food from this source, which, when both leaves were left intact, would have to be divided between the two. If, however, the cotyledons were cut off also whilst they were still functioning as sources of food for growth, then the leaf would be deprived of the food from this source and its rate of growth would be limited at a lower level. Removal of the cotyledons at a later stage in development, when they had ceased to function as a source of food for growth, would have no effect on the rate of growth of the leaf.

SUMMARY.

1. A photographic method is described for recording the daily increments in area of a leaf without detaching it from the plant; the sources of error in the method are discussed, and it is shown that they are not likely to amount to more than 1 per cent.

2. A description is given of the application of the method in a series of experiments which form the beginning of a systematic study of growth in the dwarf kidney bean (*Phaseolus vulgaris*). The results of the first two experiments in this series are given and discussed in full; the remaining experiments will form the subject of another paper.

3. When the apical bud and one first leaf were removed from each of

a number of plants the day those leaves expanded, the rate of growth of the remaining leaf was for over a week considerably faster than that of a single leaf in the case of control plants whose leaves had both been left intact.

4. When the apical bud and one first leaf were removed as before, and from some of the plants the cotyledons were likewise cut off at the same time, the rate of growth of the remaining leaf in the plants without cotyledons was for three days considerably lower than that in the case of the plants with cotyledons; after the third day, however, the two rates of growth were about the same. Removal of the cotyledons on the second or sixth day after the expansion of the first leaves had no appreciable effect on the rate of growth of the surviving leaf.

5. It is suggested that the most rational interpretation of these results would be as follows: for some days after the expansion of the first leaves, the supply of food at their disposal is limiting the rate of growth, and a considerable but decreasing portion of this supply is derived from the reserves stored in the cotyledons. Early removal of one leaf, when the cotyledons are left intact, places at the disposal of the surviving leaf a supply of food from that source which, when both leaves are left intact, has to be divided between the two. The rate of growth of this leaf is consequently increased. Early removal of the cotyledons, on the other hand, deprives the leaf of a portion of its supply of food, and results in a reduction in the rate of growth during the period when the leaf normally draws food from that source. Later removal of the cotyledons is without effect on the rate of growth of the leaf because by that time the reserves of food in those organs have been exhausted.

6. In connexion with the first of these two experiments, the problem of growth in general is discussed, and some new methods based upon a new principle are elaborated for eliminating from a series of observed growth magnitudes the daily fluctuations due to variations in external environmental conditions.

7. The form of the curve obtained by plotting actual area against time is discussed in connexion with Robertson's (9) theory and Gregory's (8) results; though the curve was of the S form and resembled that calculated by the formula (for an autocatalytic chemical reaction) suggested by Robertson as the fundamental curve of organic growth, the author supports Briggs, Kidd, and West in their view that this resemblance is merely superficial and of no fundamental importance.

8. The form of the curve of percentage increments in area is shown to be approximately of a descending logarithmic type, but it is pointed out that, as in its earlier portion this curve can be shown to be of a compound nature, the apparently logarithmic form, in this portion at any rate, is probably of little significance.

APPENDIX.

METHODS OF ESTIMATING AND ELIMINATING THE INFLUENCE OF THE DAILY ENVIRONMENTAL FACTOR.

General.

By daily factor is meant the deviation of the observed growth magnitude on a date from the 'ideal' value that would have been obtained on that date had environmental conditions remained constant at their mean for the whole period of the experiment. The object of the methods described in this Appendix is to estimate the magnitude of this daily factor on the successive days of the experiment in order that its influence may be eliminated and the 'ideal' values of the rate of growth ascertained. Three methods will be described; all are based upon the same principle, that of starting several (in this case three) identical series of plants on successive dates. The dates on which the successive periods of twenty-four hours after starting Series I ended will be denoted by the symbols D_1, D_2, D_3, &c., and their respective daily factor p_1, p_2, p_3, &c. The 'ideal' rate of growth for the first period of twenty-four hours after starting a series will be called y_1, that for the second period y_2, and so on; these will obviously fall on different dates in the different series. For example, y_1 will fall on D_1 in Series I, on D_2 in Series II, and on D_3 in Series III. The observed growth magnitude on D_1 in Series I will be a function of y_1 and of p_1, that on D_2 in Series II a function of y_1 and of p_2, that on D_3 in Series III a function of y_1 and of p_3; this provides a means for estimating the relative magnitudes of p_1, p_2, and p_3. In the same way the relative magnitudes of p_2, p_3, and p_4 may be found by comparing the observed growth magnitudes in Series I, II, and III on the dates D_2, D_3, and D_4 respectively. In this way the relative magnitudes of all the successive values of the daily factor p can be calculated, but to know their actual values it is necessary to know the mean value of the daily factor (P). The proper expression of the daily factor might be proportional to the 'ideal' value for the rate of growth, or it might be independent and additive to it; in the first case the daily factor will be that number by which the observed growth magnitude on a date must be divided to obtain the 'ideal' values for that date, in the second place it will be the number which must be subtracted from the observed magnitudes to obtain the ideal magnitudes. For example, if the daily factor p is proportional to the ideal value y the observed growth magnitudes for the first day will be y_1p_1, y_1p_2, and y_1p_3 in Series I, II, and III respectively; if the daily factor is independent and additive they will be $y_1 + p_1$, $y_1 + p_2$, and $y_1 + p_3$ respectively. In the first case, if P is the mean value of the daily factor, $y_1 \times P = y_1$ by definition, therefore $P = 1$; in the second case, $y_1 + P = y_1$ and $P = 0$. Two of the methods described, are based on the assumption that the daily factor is proportional and one on the assumption that it is additive. Of the former, one consists in a process of 'successive smoothing', and will be referred to by that name; the other is—and will be called—the 'algebraic' method. The method based on the assumption that the daily factor is additive will be termed the 'additive' method.

The Successive Smoothing Method.

As has already been mentioned, this method was tested by being applied to the correction of a series of values in a logarithmic curve that had been distorted by means of a series of arbitrary errors in the form of varying daily factors. The explanation of the method will be illustrated by means of portions of the tables compiled when making that test, but, for the sake of economy of space, only portions of the tables will be given. The values in the logarithmic curve were distorted in the manner shown in Table A.

TABLE A.

Distortion of a Logarithmic Curve by means of a Series of Arbitrary Errors in the Form of Varying Daily Factors.

		Series I.		*Series II.*		*Series III.*	
A	B	C	D	E	F	G	H
Date.	*Arbitrary Daily Factor.*	*Log. Curve.*	*Log. Curve after Distortion.*	*Log. Curve.*	*Log. Curve after Distortion.*	*Log. Curve.*	*Log. Curve after Distortion.*
D_1	1·10	50·0	55·0	—	—	—	—
D_2	1·07	40·1	43·0	50·0	53·5	—	—
D_3	0·75	32·1	24·0	40·1	30·0	50·0	37·5
D_4	0·95	25·7	24·4	32·1	30·5	40·1	38·0
D_5	1·05	20·6	21·6	25·7	27·0	32·1	33·6
D_6	1·25	16·4	20·5	20·6	25·8	25·7	32·2

The arbitrary daily factors for the dates in column A are given in column B; these represent the numbers by which the 'observed' values on those dates must be divided to obtain the 'ideal' values. These 'ideal' values are represented by the logarithmic curve which is begun on successive dates in columns C, E, and G. The numbers in columns D, F, and H represent the observed values; they were obtained by multiplying the 'ideal' values on each date by the daily factor for that date. The problem to be solved is as follows: given the observed growth magnitudes in columns D, F, and H, find the daily factors, i. e. the values in column B, and calculate the ideal values, that is to say, those in columns C, E, and G.

As a first step, the mean of the three 'observed' growth magnitudes for each day of the experiment was obtained as shown in Table B; as the first day corresponded with the dates D_1, D_2, and D_3 in the Series I, II, and III respectively, the values for those series on those dates were placed in the same line and the mean taken.

TABLE B.

Calculation of Mean Observed Growth Magnitude for each Day of Experiment.

Day of Experiment.	*Series I.*	*Series II.*	*Series III.*	*Sum.*	*Mean.*
1	55·0	53·5	37·5	146·0	48·3
2	43·0	30·0	38·0	111·0	37·0
3	24·0	30·5	33·6	88·1	29·7
4	24·4	27·0	32·2	83·6	27·9

A first approximation was then made towards the values of the daily factor in the manner shown in Table C. In column A are placed the dates D_1, D_2, D_3, &c., and in column B the three 'observed' growth magnitudes for the first day of the experiment, each opposite its respective date. Opposite these three values, in column C, their mean value is put, and in column D are placed the numbers obtained by dividing the 'observed' value on each date by the mean value. These numbers are the deviations of the three 'observed' values from their mean value. In the same way the three 'observed' growth magnitudes for the second day of the experiment are placed in column E, opposite the dates D_2, D_3, and D_4, their mean value in column F, and their deviations from that mean in column G. The corresponding values for the third day of the experiment are similarly placed in columns H, J, and K, opposite the dates

TABLE C.

Calculation of 'First' Daily Factor.

A	B	C	D	E	F	G	H	J	K	L
Date.	*Observed Value.*	*Mean Value.*	*Observed ÷ Mean.*	*Observed Value.*	*Mean Value.*	*Observed ÷ Mean.*	*Observed Value.*	*Mean Value.*	*Observed ÷ Mean.*	*First Daily Factor.*
D_1	55·0	48·3	1·138	—	—	—	—	—	—	1·1380
D_2	53·5	48·3	1·1075	43·0	37·0	1·162	—	—	—	1·13475
D_3	37·5	48·3	0·755	30·0	37·0	0·810	24·0	29·7	0·8425	0·8025
D_4	24·4	27·9	0·875	38·0	37·0	1·027	30·5	29·7	1·027	0·9763
D_5	27·0	27·9	0·968	21·6	22·0	0·982	33·6	29·7	1·132	1·0273
D_6	32·2	27·9	1·155	25·8	22·0	1·1725	20·5	16·8	1·220	1·1825

D_3, D_4, and D_5. As there were only three series, there is room for the values for the fourth day to be placed in columns B, C, and D, and those for the fifth day in columns E, F, and G, and so on for the values for each day of the experiment. The mean of the deviations of the 'observed' growth magnitudes from the mean values on each date was worked out from the values in columns D, G, and K, and is entered in column L; these mean values are the first approximations to the true daily magnitudes of the daily factor, and will be called the first daily factors. The 'observed' growth magnitudes on each date were then 'corrected' by division by the first daily factor for that date, as shown in Table D.

TABLE D.

Correction of 'Observed' Growth Magnitudes by First Daily Factors.

Date.	First Daily Factor.	*Series I.*		*Series II.*		*Series III.*	
		Observed Value.	*New Value.*	*Observed Value.*	*New Value.*	*Observed Value.*	*New Value.*
D_1	1·1380	55·0	48·25	—	—	—	—
D_2	1·13475	43·0	37·75	53·5	47·0	—	—
D_3	0·8025	24·0	30·0	30·0	37·5	37·5	46·75
D_4	0·9760	24·4	25·0	30·5	31·25	38·0	39·00

The mean of these three 'new values' for each day of the experiment is the first approximation to the true 'ideal' value of the growth magnitude for that day; it will be referred to as the first ideal value. The mean of the deviations of these 'new values' on a date from their mean values was found in the same manner as the first daily factor was found in Table C; the product of this mean and of the first daily factor for the same date is the second and theoretically closer approximation to the true value of the daily factor. The first daily factor on each date may, therefore, be regarded as the first term in the expression of the true daily factor for that date, and the second mean deviation the second term; their product will be called the second daily factor. The 'new values' in Table D were corrected by the values of the second term of the daily factor in the same way as the 'observed' values were by the first daily factor in Table D; this is obviously equivalent to correcting the 'observed' growth magnitudes by the values of the second daily factor, and the mean of the three resulting values of the growth magnitude for each day is the second ideal value for that day. By a third and fourth repetition of this smoothing process, the third and fourth terms of the daily factor and the third and fourth ideal values of the growth magnitudes were found. These fourth ideal values were found to have regained approximately the values of the true ideal curve represented by the undistorted numbers in columns C, E, and G in Table A. The distorted values, representing observed growth magnitudes, and the corresponding first, second, third, and fourth ideal values found by the four successive processes of smoothing are tabulated in Table E, where they are compared with the original undistorted values which represent the ideal values that were sought. In Graph J the undistorted logarithmic curve is compared with the mean curve of uncorrected distorted values and with the fourth ideal values. As the fourth ideal values were approximately those of the original logarithmic curve, four successive repetitions of the process of smoothing were considered sufficient when applying the process to the correction of the actual experimental data. Moreover, there is another useful check upon the accuracy of the method; if the smoothing process is performed N times, the product of the first, second, third, . . . Nth terms will be the Nth daily factor, and this will theoretically be a closer approximation than the (N – 1)th daily factor to the true value of the daily factor. When, however, the value of the Nth term becomes '1' for all dates, the Nth daily factor will be the same as the (N – 1)th daily factor, and no closer approximation to the true value of the daily factor is possible. The standard deviation, therefore, of the values of the Nth term of the daily factor on the successive days from their mean '1' is a measure of the degree to which this nearest possible approximation has been attained. This check was made use of when applying the method to the correction of the original data of Experiment I; the standard deviations of the first, second, third, and fourth terms of the daily factor in the case of plants with two leaves were found to be 1·033, 0·2663, 0·1552, and 0·1184 respectively. When testing this successive smoothing method by application to the distorted logarithmic curve, all the calculations were done by means of a slide-rule; but when applying it to the correction of the experimental data, for the sake of greater accuracy, all the calculations were done by logarithms.

As pointed out above, the nearest approximation made to the true value of the daily factor on a date will be the product of the first, second, third, and fourth terms of the daily factor for that date; this fourth daily factor is more easily calculated, however, by dividing the observed growth magnitudes on that date by the corresponding fourth ideal values. The two values of the daily factor on each date, found from plants with two leaves and from those with one respectively, have already been given in Table III, together with their mean values; they are repeated in Table F, where they are compared with the corresponding values for this factor found by the algebraic and the additive methods. The 'ideal' or smoothed values of the growth magnitudes have also been already given in Table III, and these too are repeated and compared in Table G with the corresponding 'ideal' values found by the other two methods. The two values found for the daily factor on each date by the successive smoothing method agree far more closely than the corresponding pairs of values found by either of the other two methods; the successive smoothing method was therefore adopted as the most satisfactory of the three.

TABLE E.

Test of Successive Smoothing Method. Values after Distortion and after the Successive Corrections for the Daily Factor compared with Original Values.

Day of Expt.	*Values after Distortion (Observed).*	*Values after 1st Correction (1st Ideal).*	*Values after 2nd Correction (2nd Ideal).*	*Values after 3rd Correction (3rd Ideal).*	*Values after 4th Correction (4th Ideal).*	*Original Values (True Ideal).*
1	48·3	47·3	47·57	47·95	48·33	50·0
2	37·0	38·1	38·70	38·92	39·03	40·1
3	29·7	31·7	37·78	31·67	31·57	32·1
4	27·9	26·15	25·50	25·15	25·07	25·7
5	22·0	20·8	20·21	19·91	19·78	20·6
6	16·8	15·75	15·54	15·47	15·48	16·4
7	10·7	11·7	12·02	12·19	12·34	13·2
8	8·8	9·3	9·64	9·87	10·06	10·6
9	7·4	7·7	7·89	8·04	8·09	8·5
10	6·6	6·6	6·64	6·69	6·68	6·8
11	6·1	5·8	5·73	5·69	5·62	5·5
12	5·0	4·9	4·81	4·76	4·68	4·4
13	4·1	4·05	3·95	3·88	3·82	3·5

The Algebraic Method.

Mr. Udny Yule has pointed out to me that it should be possible to calculate the 'daily factor' in a simple algebraic manner. If y_1, y_2, y_3, &c., are the 'ideal' values of the growth magnitudes on the successive days of the experiment, and p_1, p_2, p_3, &c., the values of the daily factor on the successive dates D_1, D_2, D_3, &c., and the observed growth magnitudes on each date are the products of the ideal values y and the daily factor p for that date, it is obvious that the observed growth magnitudes may be written thus:

Date.	*Daily Factor.*	*Observed Growth Magnitudes.* Series I.	Series II.	Series III.
D_1	p_1	y_1p_1	—	—
D_2	p_2	y_2p_2	y_1p_2	—
D_3	p_3	y_3p_3	y_2p_3	y_1p_3
	&c.		&c.	

From these known observed growth magnitudes it is easy to express the successive values p_2, p_3, &c., in terms of p_1; thus $y_1p_2/y_1p_1 = \frac{p_2}{p_1} = A$, $y_2p_3/y_2p_2 = \frac{p_3}{p_2} = B$, hence $\frac{p_3}{p_1} = \frac{p_2}{p_1} \times \frac{p_3}{p_2} = AB$, &c.

If 'P' is the mean daily factor for the period of the experiment, then by definition $y_1 \times P = y_1$ and $P = 1$. From this it follows that:

$$p_1+p_2+p_3+p_4+\dots+p_N = N,$$

where N is the number of the days of the experiment. Hence

$$p_1\,(1+A+AB+ABC+ABCD+\dots) = N.$$

As the values of A, B, C, &c., and of N are known quantities, the values of p_1, p_2, p_3, &c., can be calculated from this equation.

As there were three series of plants, there were two values of B, C, &c., i.e. $y_1p_3/y_1p_2 = \dfrac{p_3}{p_2} = B$, and $y_2p_3/y_2p_2 = \dfrac{p_3}{p_2} = B$; the mean value was taken, i.e. $\dfrac{y_1p_3/y_1p_2+y_2p_3/y_2p_2}{2} = B$.

The daily factor for each date was calculated separately from plants with two leaves and from those with one. These two values for each date are given in Table F, together with their mean values. The observed growth magnitudes on each date were then corrected by the mean daily factor for that date in each of the six batches of plants, and the mean 'ideal' growth magnitude for each day of the experiment found for plants with two leaves and for those with one respectively. These values are given in Table G.

TABLE F.

Comparison of the Relative Magnitudes found for the Daily Factor by Three Methods.

Date.	*Successive Smoothing Method.*			*Algebraic Method.*			*Additive Method.*		
	Plants with Two Leaves.	*Plants with One Leaf.*	*Mean.*	*Plants with Two Leaves.*	*Plants with One Leaf.*	*Mean.*	*Plants with Two Leaves.*	*Plants with One Leaf.*	*Mean.*
Feb.									
16	1·1910	1·2930	1·2420	0·7058	1·4890	1·0970	+10·89	+27·13	+19·01
17	1·3440	1·5730	1·4585	0·7912	1·6360	1·2136	+18·09	+34·23	+26·16
18	0·8980	0·9099	0·90345	0·5564	1·0160	0·7862	− 0·41	+ 0·98	+ 0·285
19	0·9822	0·9383	0·96025	0·6328	1·0010	0·8169	+ 1·74	+ 0·355	+ 1·0475
20	0·5060	0·5504	0·5282	0·3354	0·5373	0·43635	−15·26	−15·87	−15·565
21	0·8123	0·8097	0·8110	0·5534	0·7980	0·6757	− 7·835	− 8·22	− 8·0275
22	0·8480	0·8616	0·8548	0·6039	0·8656	0·73475	− 7·125	− 6·77	− 6·9475
23	1·1330	1·1600	1·1465	0·8690	1·1950	1·0320	− 1·60	− 0·945	− 1·2725
24	1·2710	1·4620	1·3665	1·0810	1·5700	1·3255	+ 1·75	+ 3·755	+ 2·7525
25	1·0420	1·0420	1·0420	0·9725	1·0450	1·00875	+ 0·25	− 1·750	− 0·750
26	0·9419	0·9740	0·95795	0·9183	0·9456	0·93195	− 0·775	− 3·95	− 2·3625
27	0·3370	0·3942	0·3656	0·3590	0·2862	0·3226	− 4·700	− 9·00	− 6·85
28	1·0400	1·0350	1·0375	1·2960	0·8513	1·07365	− 0·173	− 4·45	− 2·3125
Mar.									
1	1·6030	1·2980	1·4505	2·1840	1·2340	1·7090	+ 0·290	− 1·92	− 0·8250
2	0·6200	0·4620	0·5410	0·9445	0·4218	0·68315	− 0·800	− 6·05	− 3·425
3	1·1300	0·9779	1·05395	1·9720	0·9353	1·45365	+ 1·325	− 3·05	− 0·8625
4	1·2940	1·2570	1·2755	2·2230	1·1630	1·6930	+ 1·725	− 2·82	− 0·5425

TABLE G.

Mean daily percentage Increments in Area after 'Correction' by the Mean Daily Factors found by the Three Methods.

Day of Experiment.	*Successive Smoothing Method.*		*Algebraic Method.*		*Additive Method.*	
	Plants with Two Leaves.	*Plants with One Leaf.*	*Plants with Two Leaves.*	*Plants with One Leaf.*	*Plants with Two Leaves.*	*Plants with One Leaf.*
1	46·89	56·33	54·41	65·43	41·08	52·45
2	40·07	44·69	47·02	52·26	34·73	41·63
3	34·99	40·07	41·22	47·34	33·18	36·83
4	31·07	33·02	37·17	39·46	30·91	32·96
5	20·38	24·45	24·26	29·14	28·70	27·88
6	16·79	19·65	19·40	22·78	21·30	24·08
7	13·37	16·29	14·81	17·44	17·74	19·64
8	11·22	11·49	11·86	12·16	13·02	13·49
9	9·36	11·32	8·93	11·65	9·02	12·87
10	7·64	10·35	8·16	11·01	9·22	11·55
11	6·39	6·86	6·62	7·08	9·02	9·54
12	4·54	7·35	4·39	7·73	7·945	9·69
13	5·40	4·66	4·61	4·06	7·35	6·99
14	3·88	4·78	3·06	3·74	5·65	6·37
15	3·70	3·76	2·79	2·85	5·19	5·41
16	2·02	2·98	1·49	2·23	3·05	4·225

The Additive Method.

This method is based on the assumption that the observed growth magnitude on a date is the sum of the 'ideal' growth magnitude and of the daily factor for that date; i.e. it assumes that the observed growth magnitudes on successive days of the experiment in Series I are y_1+p_1, y_2+p_2, &c., those in Series II y_1+p_2, y_2+p_3, &c., those in Series III y_1+p_3, y_2+p_4, &c. It has already been shown that, if P is the mean daily factor for the period of the experiment, $P = O$; for, by definition, $y_1+P=y_1$. The relative values of p_1, p_2, p_3, &c., can be found, for $(y_1+p_1)-(y_1+p_2)=p_1-p_2$ and $\dfrac{(y_1+p_2)-(y_1+p_3)+(y_2+p_2)-(y_2+p_3)}{2}=p_2-p_3$, &c., and (y_1+p_1), &c., are known values. The absolute values of p_1, p_2, &c., can be found also, because P, that is $\dfrac{p_1+p_2+p_3+\ldots+p_N}{N}, = O$.

The daily values of 'p' were again found separately from plants with two leaves and from those with one. They are given, together with their mean values, in Table F. When 'correcting' the observed growth magnitudes by the mean daily factors found by this method, the values of the latter have to be subtracted or added according to their sign. The ideal growth magnitudes obtained in this way are given in Table G.

Relative Reliability of the Three Methods.

The two values of the daily factor found for each date from plants with one leaf and those with two leaves respectively should theoretically be identical. In the case of these pairs of values found by the successive smoothing method there is a very fair agreement; consequently considerable reliance may be placed on the accuracy of the values of the daily factor and of the 'ideal' growth magnitudes found by that method. The two values found for the daily factor on each date by the algebraic method are widely divergent; consequently little reliance may be safely placed on the values found by that method. When that method was applied, however, to the correction of the distorted logarithmic curve, the values of the daily factor and the ideal values of 'growth magnitudes' were obtained with considerable accuracy; consequently the divergences that occurred when it was applied to the correction of the experimental data were probably due to the influence of the irregularities that are inherent in all biological data. In the case of the distorted logarithmic curve, the three 'observed' growth magnitudes for the first 'day of the experiment' are, in actual fact, y_1p_1, y_1p_2, and y_1p_3 in Series I, II, and III respectively; whereas, in the experimental data, they will be $(y_1+a)p_1$, $(y_1+b)p_2$, and $(y_1+c)p_3$ respectively, where a, b, and c are small divergences from the ideal value of y_1 due to variations inherent in all living organisms. It seems that these sources of error, a, b, and c, have a greater distorting effect on the results when the data are corrected by the algebraic method than when they are corrected by the successive smoothing method. The two values found for the daily factor for each date by the additive method are widely divergent, and the resulting curves of 'ideal' growth magnitudes are more irregular and less convincing than the original uncorrected curves of growth. These divergences may be due in part to the same cause as those arising in the algebraic method, but it seems probable that they are in part due to the hypothesis upon which the method is based being false; it seems probable that the proper expression of the growth magnitude is yp and not $y+p$.

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The Spermatogenesis of Anthoceros laevis, L.

BY

KRISHNADAS BAGCHEE,

Palit Scholar, University of Calcutta.

With Plate VI.

INTRODUCTION.

THE spermatogenesis in *Anthoceros* has been broadly treated by Campbell (1), who, owing to the minuteness of the spermatogenous tissues, gives little cytological detail. Professor Farmer suggested that a detailed study of the spermatogenesis in *Anthoceros* might throw some light on the affinities of the Anthocerotales to other members of the Hepaticeae. This investigation was accordingly taken up at his suggestion, and the writer wishes to express thanks for his advice and guidance during the course of the investigation.

METHODS.

A part of the material was fixed in acetic alcohol in the field, other material was kept in the laboratory growing on large sods under a bell-jar, and was fixed from time to time in various strengths of chromo-acetic, Flemming's, and Hermann's solutions. Chromo-acetic of medium strength and Flemming's weak solution proved quite good, but much better results were obtained from material fixed in half-strength Hermann's fluid for ten hours. The material was very quickly taken through grades of xylol to paraffin, so that the time taken for the entire process up to embedding was reduced to six hours. Even then great difficulty was experienced in cutting sections, as the hot xylol makes the antheridia very hard and brittle; sections were cut from 3 to 8 μ. The process of division of the spermatogenous tissues has been studied from sections of 4 to 6 μ and the development of the spermatozoids has been described from sections of 3 μ. In staining, Flemming's triple stain, polychrome-methylene blue, and Heidenhain's iron-haemotoxylin, followed by Bismarck-brown (1 per cent.

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Bismarck-brown in 15 per cent. alcohol) as counter-stain, were used. The haematoxylin was carefully washed away, leaving the delicate cell-wall colourless, when a careful touch of Bismarck-brown gave a satisfactory contrast. The cell-wall took a bright brown colour, while the cytoplasm, mucilaginous layer, and the pectic membrane separating the spermatozoids took a much lighter colour with just a shade of difference between them. The figures are all drawn from Heidenhain-stained preparations, but the results were all checked by Flemming's and polychrome-stained preparations.

THE NATURE OF THE SPERMATOGENOUS TISSUES.

The cells of the spermatogenous tissues are seldom seen to divide simultaneously, though in some mature antheridia one finds several stages of development. This tissue is arranged in the antheridia in form of a regular pyramidal cone or a rectangular patch, obviously related to the early segmentation of the antheridium during the metaphase and telophase divisions. The spermatogenous cells present a more or less uniform stage when they advance towards the formation of spermatocytes. The antheridium attains its mature size before the formation of spermatocytes from the spermatogenous tissue is complete. The cells of the spermatogenous tissue divide very rapidly, with the result that the spermatid mother-cells, which are packed in the form of regular cubical cells, decrease to almost one-fourth of the original size. The cells of the antheridia retain their cubical shape throughout the division. The amount of mucilaginous substance in the cells increases as the cells advance towards the final divisions leading to the formation of the spermatocytes.

THE PROPHASE IN THE ANTHERIDIAL NUCLEAR DIVISIONS.

The resting nucleus of the young antheridial cell contains finely granular cytoplasm and a large and prominent nucleolus which is rather eccentrically situated (Pl. VI, Fig. 1). Several highly refractive granules are noticed outside the nuclear membrane. The nuclear membrane is at first very fine and almost hyaline, but it becomes considerably thicker as the nucleus advances towards the spireme stage (Fig. 2). The cytoplasm becomes much coarser during the early prophase, and there is a gradual accumulation of granular chromatin which later on takes the form of a delicate thread (Fig. 2). This is very clear in a thick tangential section which shows the chromatin thread in the form of a concave reticulum along the nuclear membrane, while a thin median section shows only the cut ends of the reticulum along the membrane. The thread is drawn out at several points in the nuclear area. This concentration of chromatic material gradually increases at those points, so that after a time there appear several thick knots within the nuclear space (Fig. 3). These knots are connected with the nucleolus by fine filaments, while a number of loose ends radiate from the other side of the knots towards the nuclear membrane. The nucleolus in the meanwhile loses its round shape and appears to be rather elongated (Fig. 3); and this phenomenon might be due to the pull of the chromatin reticulum. Soon after this the whole mass of chromatin fragments into four distinct groups (Fig. 4), which finally give rise to four slightly curved, rod-shaped chromosomes of two different sizes, two large and two small (Fig. 5).

THE NATURE OF THE SPINDLE.

The spindle is organized by numerous delicate fibrils which slowly accumulate along the nuclear membrane during the prophase. Neither polar caps nor any specialized part of the cytoplasm was observed to be connected with its formation. The spindle is very regular in the early stages of division, and its axis coincides with the long axis of the cell (Fig. 5), as in an ordinary division. The threads of the spindle are clear, but the poles generally vary in shape from sharply pointed cones to flat ones in which the fibres run almost in parallel bundles. The indication of the advance division is given by the oblique position of the spindles. The final division of the spermatid mother-cells, which are very small, is easily recognized by the greater number of oblique spindles, though even here a great many of them are in the long axis of the cell, and not oblique to it (Fig. 8). But cases are also seen (Fig. 9) where the finally dividing cells have all the spindles arranged diagonally, and this has been described by many authors as characteristic of the final division in the spermatogenesis of the Hepaticeae. Unlike the earlier spindles those of the final division have sharp-pointed poles. Granules are noticed at the poles, but, being of very rare occurrence, have been omitted in the diagrams.

TELOPHASE AND THE FORMATION OF SPERMATIDS.

The chromosomes lose their individuality as soon as they reach the poles, where they are reduced to a narrow, crescent-shaped band of chromatin (Fig. 10). The spindle fibres become much finer and ultimately break away in the middle, and the two parts recede towards the poles, so that a clear space is formed at the equatorial region (Fig. 11). As the result of this peculiar kind of division no actual cell-wall is formed between the two daughter nuclei, and the spermatids are only separated by a delicate pectic membrane. The nucleus keeps the elongated shape of the original chromatin for a short time, but it rounds itself off and appears as a faintly-stained body when the spermatids undergo a period of rest. The young spermatids (Fig. 12) have a very characteristic appearance. They present either a triangular or a semicircular outline, depending on the original position of the spindle, i. e. whether the latter was oblique or longitudinal in position. The nucleus always lies near the broadest side of the spermatids which are facing each other. The spermatid contracts away from the cell-wall, leaving a clear space all round, which is filled up by more hyaline mucilaginous substance. The cell-wall undergoes the greatest degree of mucilaginous disintegration at this stage, and the middle lamella is almost indistinguishable.

The origin of the mucilaginous substance and its gradual increase from the early divisions to the formation of spermatids are not only due to the disintegration of the cell-wall but also to the metabolic activity of the young spermatids, which seems to a certain extent responsible for its formation.

BLEPHAROPLAST AND THE FORMATION OF SPERMATOZOID.

Special attention was given to the exact processes concerned in the formation of blepharoplast and of spermatozoids, which varies considerably in the Hepaticeae; even in the case of a single species such as *Marchantia polymorpha* there is a difference of opinion, and consequently controversy has arisen. Unfortunately the present material does not provide much scope for a detailed study of the spermatozoid formation. Repeated attempts were made to detect the centrosome or any structure like a centrosome, but without any success. It has been already pointed out by Davis (2) that centrosomes are entirely absent in *Anthoceros*. The blepharoplast arises as the result of fragmentation of the main mass of chromatin, and it lies for a short time at one end of the narrow strip of chromatin (Figs. 15 and 16). The blepharoplast thus appears rather late in the history of spermatogenesis of *Anthoceros*, and it disappears as soon as the cilia are visible around the young spermatozoid. The spermatid has now changed to an almost circular shape, and the nucleus is reduced to a faintly-stained body (Fig. 17). The nucleus lies eccentrically in the spermatid. The body of the spermatozoid is formed by the elongation of this nucleus and by further condensation of nuclear material along the outer contour of the spermatid (Figs. 18 and 19). The cell-wall is gradually dissolved, while the membrane keeps longer intact. Finally, the membrane is lost sight of as the spermatozoids become embedded in a loose matrix of mucilaginous substance and occupy a quarter of the antheridial cavity. The mature spermatozoid (Fig. 20) has a curved and linear body with a slightly club-shaped head. The cilia are almost of the same length as the body of the spermatozoids.

DISCUSSION.

There have been a considerable number of investigations on the development of the spermatozoids in the Hepaticeae, and they all tend to show that the final division of the spermatid mother-cell conforms to the diagonal type. In *Anthoceros*, however, the final spindle is both oblique as well as longitudinal, and these two arrangements occur side by side and both give rise to spermatids.

The origin and function of blepharoplast are two interesting points in the history of the spermatogenesis. Ikeno (6) first pointed out the presence of centrosomes in the cells of the spermatogenous tissues of *Marchantia polymorpha*. According to his very careful investigations they appear in the later dividing cells of the antheridia, and are always present during the final division of the spermatid; the single centrosome migrates to the acute angle of the spermatid, and forms the blepharoplast from which the cilia arise. Ikeno points to the probable homology of the two organs—the centrosome and the blepharoplast—in a phylogenetic sense. The existence of centrosomes in *Marchantia* and their subsequent change to blepharoplasts, the cilia-bearers, has been denied by Mottier (8) and more recently by Escoyez (4). Escoyez maintains that the corpuscles which appear in the spermatogenetic cells during the early divisions, and which can be traced from one division to another, are mere cell-structures, and are not genuine centrosomes, while he argues that the others, which always appear in the final and diagonal divisions, are really blepharoplastic in nature. Lewis (7) regards these bodies as centrosome-like rather than true centrosomes, but he seems to favour Ikeno's (6) view of the homology of the two organs in question.

It is out of place here to go farther into the details of this controversy, as the centrosome is entirely absent in *Anthoceros*. Several slides were obtained which showed the 'aster' and 'diaster' stages of the final division (Figs. 7, 9, and 10), which, according to Ikeno, are the most critical stages where the centrosomes are invariably present in *Marchantia*, but even at these stages persistent search failed to reveal any structure which could be positively identified as a centrosome. In this respect *Anthoceros* behaves much like *Fossombronia longiseta*, where Humphrey (5) could not trace the origin of blepharoplast from a centrosome. But in *Anthoceros* the origin of blepharoplast, at any rate, which has not hitherto been clearly seen, and has up till now been a subject of speculation, is very clear. It is formed by fragmentation from the main mass of chromatin of the nucleus. Even in the same slide one can find all the stages from its beginning in the main body of the chromatin (Figs. 13 and 14) to the lateral migration of the small fragment as a blepharoplast (Figs. 15 and 16), while the bigger body rounds itself off and takes the central position as the nucleus (Fig. 17).

It is interesting to note that the origin of blepharoplastic corpuscles in the spermatids of *Anthoceros* shows some similarity to the formation of the 'chromatin bodies' which have been observed in animals and plants, both in vegetative and reproductive cells. The history of the development of these bodies has been studied in *Galtonia candicans* by Miss Digby (3).

They originate either from the nuclear framework or from the nucleolus. They may lie in the nuclear cavity or they may invade the neighbouring cells where they remain in the cytoplasm. But the further history of blepharoplastic corpuscles in *Anthoceros* is not so simple as that of the 'chromatin bodies' of the higher plants. In *Anthoceros* and in other members of the Hepaticeae they are always connected with the formation of the motile organs (cilia), while in *Galtonia* and in other higher plants they are destined to undergo an extremely rapid degeneration.

The contraction of the chromatin reticulum as knots at several points of the delicate spireme in the evolution of chromosomes is a regular phenomenon in all the successive divisions (Figs. 3, 4, and 8). The number of chromosomes is four, which agrees with the number given by Davis (2).

SUMMARY.

1. The gametophytic number of chromosomes is four.

2. The centrosome is entirely absent in the spermatogenesis of *Anthoceros*.

3. The final division of the spermatid mother-cell is both oblique as well as regular, and the two kinds of divisions take place side by side and both give rise to the spermatocytes.

4. No true cell-wall is formed between the spermatids, which are separated by a hyaline membrane of pectic substance.

5. The blepharoplast arises from the main body of chromatin as the result of fragmentation. It disappears as soon as the cilia are visible round the spermatid.

6. The spermatozoid has a linear body which is a little broader towards the head, with the cilia of almost of the same length as the body.

BOTANY DEPARTMENT,
IMPERIAL COLLEGE OF SCIENCE,
SOUTH KENSINGTON.

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EXPLANATION OF PLATE VI.

Illustrating Mr. Krishnadas Bagchee's paper on the Spermatogenesis of *Anthoceros laevis*, L.

All the figures, with the exception of Figs. 20 and 21, were drawn with the camera lucida under 1/15 semi-apochr. Koristka with comp. oc. 18 (× 2550).

Figs. 20 and 21 were drawn with camera lucida under 2 mm. apoc. imm. Zeiss N.A. 1·40 with comp. oc. 18 × 2250.

Fig. 1. Resting stage of nucleus in the cells of spermatogenous tissue of very young antheridium.

Fig. 2. Delicate spireme stage of nucleus of almost the same age as Fig. 1.

Fig. 3. Formation of thick spireme from several knots.

Fig. 4. Fragmentation of spireme into four groups of chromatin.

Fig. 5. Aster stage of mitosis of very young antheridium.

Fig. 6. Diaster stage of almost the same age as Fig. 5.

Fig. 7. Spireme stage of nucleus from which the spermatid mother-cells arise.

Fig. 8. Final division in spermatid mother-cells showing mixed spindles.

Fig. 9. Final division with oblique spindles.

Fig. 10. Anaphase stage of mitosis in spermatid mother-cells.

Fig. 11. Late telophase of final division, showing the formation of clear space in the equatorial region of the spindle.

Fig. 12. The resting spermatids within the spermatid mother-cells.

Fig. 13. The appearance of constriction in the main mass of chromatin after the late telophase.

Fig. 14. Formation of blepharoplast.

Figs. 15 and 16. Lateral migration of blepharoplast.

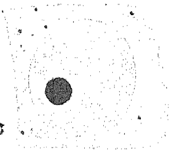
Fig. 17. Lateral migration of blepharoplast and the formation of nucleus by rounding off of the main mass of chromatin.

Fig. 18. Formation of cilia and disappearance of blepharoplast.

Fig. 19. Condensation and elongation of spermatozoids.

Fig. 20. A later stage in the growth of spermatozoids.

Fig. 21. A free spermatozoid.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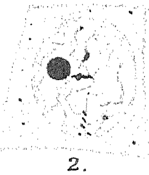
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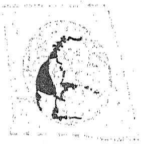
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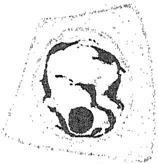
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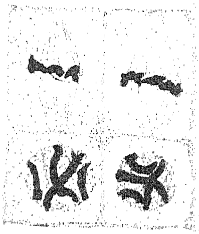
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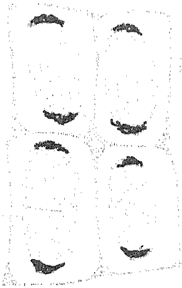
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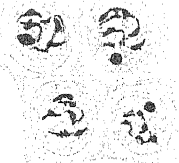
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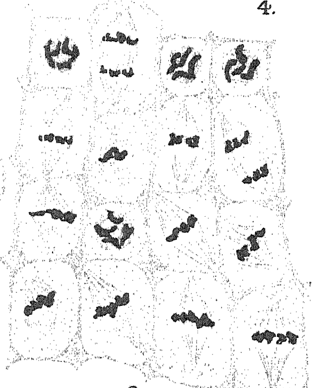
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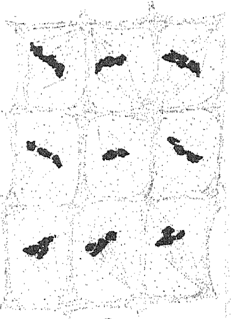
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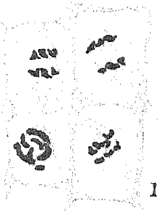
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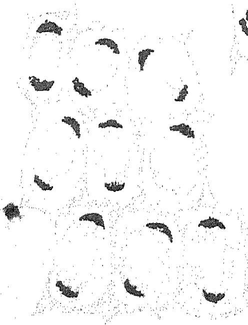
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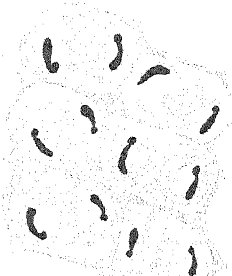
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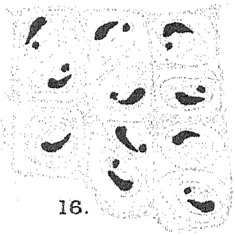
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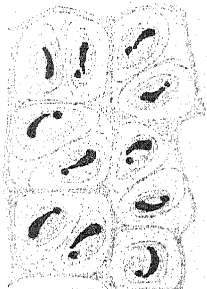
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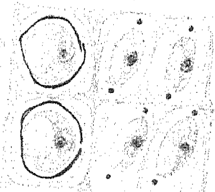
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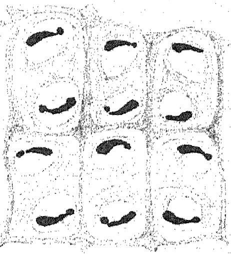
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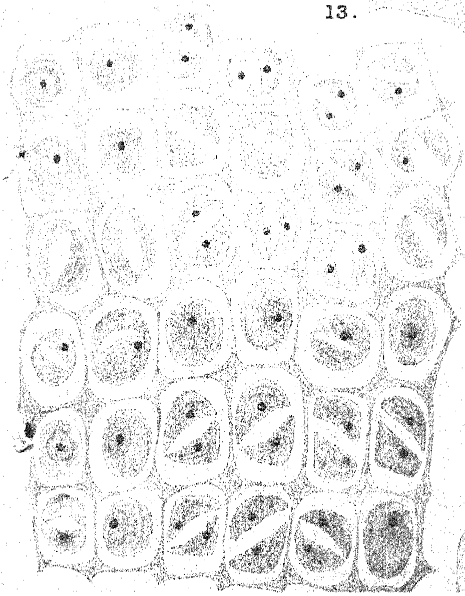

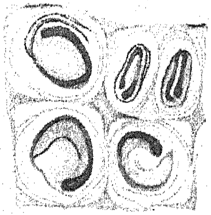
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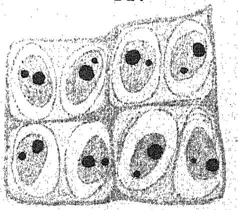
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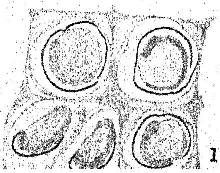
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Studies in the Physiology of Parasitism.

X. The Growth Reactions of Certain Fungi to their Staling Products.

BY

C. BOYLE

(*Department of Plant Physiology and Pathology, Imperial College of Science and Technology, London*).

With five Figures in the Text.

INTRODUCTION.

NUMEROUS investigations have been recorded dealing with the influence of various chemical substances on the germination and growth of fungi, but comparatively few observations have been made on the effect on the growth of fungi of the products of their own metabolism.

Fungi which develop for some time on solid or liquid media sooner or later show a marked diminution in rate of growth, varying with the organism and the medium used. When considering the various factors which contribute towards such a diminished rate, the first which suggests itself is the exhaustion of the food materials; changes which manifest themselves in an increase or decrease of acidity or alkalinity must also be taken into account. Finally, organisms, during their growth, liberate materials about the chemical properties of which little is known.

The object of the present investigation was to trace, step by step, the staling of a solution and then to attempt to discover by what means and to what extent the capacity for growth could be recovered. This is a necessary preliminary to an attempt to determine the chemical nature of the staling products.

HISTORICAL.

Nikitinsky (6) investigated the influence of the metabolic products of moulds on their own development. He grew *Penicillium glaucum*, *Penicillium griseum*, *Mucor stolonifer*, *Aspergillus flavus*, *Saccharomyces rosaceus*, and *Saccharomyces cerevisiae* in flasks, using an ordinary synthetic medium. The mycelium produced was removed after 10–20 days

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and its dry weight determined. The liquid remaining in the flasks was sown with spores of the same (or a different) species, left for a further period of 10 to 20 days, and the mycelium removed and the dry weight determined as before. This was repeated until the liquid remaining in the flasks gave no further growth. He also tried the effect of the addition of nutrient, or of acid or alkali, to the partially staled medium. By varying the source of nitrogen in his culture medium he obtained very different degrees of staling. When ammonium chloride was used as the source of nitrogen, inhibition of growth set in very quickly and the medium became acid. The NH_3 was apparently removed and the HCl left behind soon rendered the medium unsuitable for further growth. By neutralizing the acidity by the addition of alkali, comparatively good growth was obtained. When ammonium tartrate was substituted for ammonium chloride, the NH_3 was utilized as a source of nitrogen and the tartaric acid radicle as a source of carbon. In this case there was less acidity developed and the medium became less stale. When peptone was used as the source of nitrogen and carbon the medium became alkaline, but the alkalinity was developed more quickly in cultures of *Penicillium* sp. than in those of *Aspergillus niger*. In the case of the latter the NH_3 was neutralized by the oxalic acid produced. In this connexion the author states: 'After the addition of KH_2PO_4 to the culture liquids we find comparatively good development, that is, the inhibition of growth was caused by the alkaline reaction only.' Here, as in other parts of this author's work, the evidence brought forward is not sufficient to justify the conclusion. When glucosides were introduced into the medium as the source of carbon, Nikitinsky obtained staling effects which were not due to acidity and of which he was unable to determine the cause. Cases are also recorded where a certain amount of fungal growth accelerates the growth of another fungus when sown afterwards in the same liquid. Such stimulating effects were often masked by the presence of inhibiting factors.

In a preliminary communication on the germination and development of spores of fungi, Küster (3) noted that the inhibiting effect of stale solutions on fungal growth could be partly removed by boiling. He grew *Aspergillus niger* in a plant decoction for nine months, and at the end of that time took a sample of the stale liquid and boiled a portion. Hanging-drop cultures of the boiled and unboiled liquids were set up and inoculated with fresh spores of the same fungus. After two days there was no growth in the unboiled portion, whereas long vigorous germ-tubes were produced in the boiled portion at the end of twenty-four hours. He also observed that the same result was obtained when the stale medium was inoculated with the spores of a different fungus from that which produced the staling.

Lutz (5) grew various mould fungi in nutrient solutions of different composition, and found that in the course of their development they gave rise to substances which retarded or stimulated their own growth. He was unable to identify any of these substances, but found that with enzymes they shared the property that at high temperatures they were destroyed or at least temporarily deactivated. In the case of some stale solutions after passage through a porcelain filter the growth-retarding or growth-accelerating substances were no longer demonstrable in the filtrate; in the case of other stale nutrient solutions these substances were not held back by the filter. The retarding or stimulating effects were not confined to the fungi by which these substances were produced; they also affected the spores of other fungi.

Wehmer (9) found that *Penicillium* sp. grew badly on a nutrient consisting of sugar and ammonium sulphate. When potassium nitrate, ammonium nitrate, ammonium chloride, ammonium malate, ammonium citrate, ammonium tartrate, also peptone and asparagin, were used as the source of nitrogen, normal growth resulted. The cultures containing ammonium sulphate as the source of nitrogen developed strong acidity which ultimately resulted in the death of the fungus. Similar results were obtained in cultures containing ammonium nitrate and ammonium chloride, but not when potassium nitrate was used. These results were not obtained when *Aspergillus niger* was grown under the same conditions.

Zeller and Schmitz (10) grew various fungi in mixed cultures in order to determine whether the inhibition or stimulation of growth, as the case may be, might not be the result of the depletion of the available carbohydrates in the medium or a change in the hydrogen-ion concentration. Agar plates were each inoculated with three of the fungi in such a way as to have all possible combinations of each fungus, and from these the reciprocal influence of growth was determined. In cases where there was a marked inhibition or stimulation of growth between two fungi on the plates, these were afterwards grown on sand slopes to which the same ingredients as were used in the agar plates were added. After considerable growth had been made by the first fungus the solution was filtered off, sterilized, and inoculated with the reciprocal fungus. The amount of growth resulting from the second inoculation was determined by ascertaining the dry weight of the fungus mat produced, and the amount of sugar remaining in the solutions was estimated. The authors were unable to establish any definite correlation between the carbohydrate content of the nutrient solution and the growth of the second fungus. As recognized by the authors, the method is open to a very serious objection, in that any toxic substances produced by the first fungus might be destroyed in the process of autoclaving between the first and second inoculation, and misleading results might thus be obtained. The hydrogen-ion concentrations of the solutions were determined after the fungi had grown in them for two weeks, and the results obtained showed that there was no definite relation between the active acidity produced by these fungi and their ability to inhibit or stimulate the growth of another.

In the course of an investigation on the growth of wood-destroying fungi on liquid media, Zeller, Schmitz, and Duggar (11) found that under the conditions studied the fungi investigated showed a general tendency to increase the active acidity of the medium during growth. They concluded, however, that the hydrogen-ion concentration of the medium did not appear to be the limiting factor in growth.

Boas (1) observed that *Aspergillus niger*, when grown on a nutrient medium containing maltose (5 per cent.) and urea (2 per cent.), produced such quantities of ammonia as to cause the death of the fungus. Other fungi, such as *Botrytis cinerea* and *Oidium*, grown under similar conditions remained alive for months.

METHODS.

Previous work carried out in this laboratory has shown that a species of *Fusarium* originally isolated from apples possessed very active staling properties. Preliminary trials were also made, using *Botrytis cinerea*, but it was found that the latter fungus staled comparatively slowly on the media used; later work was therefore confined to *Fusarium* sp. Stock cultures of the fungus were grown on potato mush agar. The medium was prepared by weighing 200 grm. of washed potatoes, cutting into small pieces about 1 cm. cube and steaming for two hours in a litre of water. The whole was then strained through muslin and 15 grm. agar per litre added to the extract. Tubes were poured, sterilized, and slanted in the usual way. Several media were tried for stock culture purposes, but this proved to be the most suitable one for the production of spores, an abundant supply of which was required for the inoculations to be described.

As liquid media for the growth of *Fusarium* sp., Richards' solution (7) was selected as representing an ordinary synthetic culture medium and potato and apple extracts as representing plant decoctions. The potato extract was prepared as described above, without the addition of agar. Apple extract was prepared by adding to 200 grm. of apple, cut up into small pieces, one litre of water and steaming for two hours. The whole was then strained through muslin and the extract allowed to stand until any remaining solid had settled to the bottom, when the supernatant liquid was siphoned off.

The cultures were set up in 500 c.c. conical flasks. In order that the conditions of germination and growth as regards depth and surface area of culture medium might be as uniform as possible, the flasks used throughout this work were specially selected for their uniformity. To each flask was added 50 c.c. of the medium to be used. They were then plugged with cotton-wool and sterilized at 120° C. for twenty minutes. A concentrated spore suspension of *Fusarium* sp. was prepared in sterile distilled water, and twenty drops (= 1·1 c.c.) added to each flask by means of a sterile pipette. The flasks were incubated at 20° C.

As observed by previous workers in respect of other species of fungi, it was found that the inhibiting action of *Fusarium*-staled medium was not confined to that genus, but also extended to other genera. For example, a medium on which *Fusarium* sp. had been growing for some time was found to exert a greater retarding influence on spores of *Botrytis cinerea* than on spores of *Fusarium* sp. Flasks of nutrient inoculated with *Fusarium* sp. as described above were taken at intervals, depending on the rate of staling on the particular medium, and filtered under sterile conditions. Since the progress of staling was to be measured by the effect of the stale liquid on the germination and growth of fresh spores, the presence in the filtrate of spores produced by the mycelium in the stale medium would be objectionable if fresh spores of *Fusarium* sp. were used in testing the germinative capacity of the stale liquid. The use of spores of *Botrytis cinerea* for this purpose instead of those of *Fusarium* sp. appeared to possess several advantages. *Botrytis* spores are much more susceptible to the metabolic products of *Fusarium* sp. than are spores of the original fungus producing the products; consequently the staling effect is obtained much sooner. They have the further advantage that the germ-tubes of *Botrytis* spores can be measured microscopically with much greater accuracy than can those of *Fusarium*. In the case of the latter it is often difficult to tell where spore ends and germ-tube begins.

For these reasons the progress of staling was measured throughout this work by the effect of the stale liquid on the germination and growth of spores of *Botrytis cinerea*.

The stock cultures of the fungus were grown on potato mush agar. The *Botrytis* spore suspensions used for inoculation were prepared from cultures ten to fourteen days old and centrifugalized several times. Two c.c. portions of the various liquids whose germinative capacity was to be tested were placed in sterile test-tubes and one drop of a concentrated suspension of spores of *Botrytis cinerea* added. Drop cultures of each liquid were then transferred to two clean slides—three drops to each—and the slides placed in sterile Petri dishes. The atmosphere in the Petri dishes was kept moist by placing damp blotting-paper underneath the lids. The cultures were incubated at 20° C. After ten to fifteen hours the cultures were examined and the length of germ-tube produced by the spores was measured.

It was observed that considerable differences may occur in the capacity for germination and growth of individual spores produced under similar conditions. This is particularly the case in any medium which does not produce vigorous germination. In order to eliminate experimental errors arising out of differences in size of the drop cultures, pipettes of similar calibre were used throughout. Usually 50 to 100 germ-tubes were measured in each culture, depending on the amount of variation in growth of the individual spores.

Generally all the spores in a single field in each drop were measured so that the average represents measurements taken from six different fields for each culture. By calculating the probable error[1] of the average we are enabled to interpret the significance of the differences between the various averages.

The criterion of average germ-tube length was found to be a much better index of the suitability of a medium for growth than percentage germination. Except in the most unsuitable media, practically all the spores of *Botrytis cinerea* germinate in ten to fifteen hours, but the differences in the rate of growth are very marked.

In order to compare the amount of growth of *Fusarium* sp. on the three media used, and also to find whether there was any correlation between the rate of staling on a particular medium and the amount of fungal growth, the weight of the mycelial mat produced was determined in each case. The mycelium was filtered off, the filter-paper having been previously dried over $CaCl_2$, weighed, and sterilized. When the stale liquid had drained away, the mycelium was well washed with distilled water, dried over $CaCl_2$, and weighed again.

The hydrogen-ion concentration of the staled and control solutions was determined by the colorimetric method as elaborated by Lubs and Clark (4). The standard buffer solutions were prepared from pure chemicals recrystallized several times and made up with distilled water. The colorimetric comparisons were made with the aid of the so-called 'comparator' of Hurwitz, Meyer, and Ostenberg, the procedure being as follows: To a test-tube containing 5 c.c. of the test liquid was added a definite amount of the appropriate indicator, and the colour developed compared with the colours obtained when the same amount of the indicator was added to equal quantities of the standard buffer solutions. Behind the test liquid stood a tube of distilled water. The tubes standing behind the buffer solutions contained the test liquid without indicator, so that equal quantities of the same liquids were being viewed in all cases.

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[1] In order to get some idea of its magnitude, the probable error of the mean has been calculated in a few instances in which the variations were perhaps greater than normal. In one case where 157 germ-tubes were measured, the average worked out at 3·0, and the probable error at 0·09, or 3 per cent.

The indicators used were those recommended by Lubs and Clark, giving a range from pH 1·2 to pH 9·8.

Various methods were adopted to discover by what means and to what extent the capacity of sustaining growth could be restored to staled solutions as a preliminary to a chemical examination of the staling products. As already mentioned, the first factor to be considered in examination of a staled solution is the extent to which inhibition of germination and growth is due to exhaustion of the food-supply. This was tested by adding to the staled liquid varying proportions of the original unstaled nutrient—controls to which sterile distilled water was added being set up at the same time to find out the effect of dilution on the toxic substances present.

When the reaction of the staled medium differed considerably from that of the control, HCl or NaOH, as the case may be, was added so as to restore the pH of the staled medium as near as convenient to that of the control.

In order to determine whether the toxic effect of staled solutions might not be due to the presence of thermolabile substances, portions were boiled, usually to about half the volume, and the boiled portion made up to the original volume with sterile distilled water. Portions of the boiled liquid were taken and corrected for pH as before by the addition of HCl or NaOH.

Attempts to restore the germinative capacity to staled media by other means will be described later.

Care of glassware. All tubes and slides used were first cleaned in bichromate-sulphuric acid mixture, after which they were repeatedly washed with distilled water. The test-tubes and pipettes were thoroughly washed and sterilized after each experiment.

Experimental data. In order that the germ-tube measurements in the various series of experiments might be comparable one with another, the average germ-tube length in the control nutrient media is reckoned as twelve divisions[1] on the ocular micrometer and all other measurements are calculated accordingly.

The following abbreviations are used throughout the various tables: *R.S.* = Richards' solution; *A.E.* = apple extract; *P.E.* = potato extract; $R.S.F._x$ = Richards' solution in which *Fusarium* has been growing for 10 days.

Exhaustion of food materials. Apple extract prepared as described was inoculated with *Fusarium* sp. and incubated at 20° C. for seventeen days. The mycelium was then filtered off under sterile conditions, and to the staled liquid were added various proportions of unstaled apple extract in one case, and sterile distilled water in the other. The cultures were inoculated with spores of *Botrytis cinerea*, incubated at 20° C. for eight hours, and the germ-tube length measured.

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[1] One division = 12 μ.

TABLE I.

Series A. Dilution with apple extract.

	A.E.F.$_{17}$	1 *pt. A.E.F.*$_{17}$ 1 *pt. A.E.*	1 *pt. A.E.F.*$_{17}$ 9 *pts. A.E.*	1 *pt. A.E.F.*$_{17}$ 39 *pts. A.E.*	1 *pt. A.E.F.*$_{17}$ 99 *pts. A.E.*	*A.E.*
Average germ-tube length	0	0	7·5	8·0	9·9	12·0

Series B. Dilution with water.

	A.E.F.$_{17}$	1 *pt. A.E.F.*$_{17}$ 1 *pt. Water.*	1 *pt. A.E.F.*$_{17}$ 9 *pts. Water.*	1 *pt. A.E.F.*$_{17}$ 39 *pts. Water.*	1 *pt. A.E.F.*$_{17}$ 99 *pts. Water.*	*Water.*
Average germ-tube length	0	0	1·7	4·5	5·2	0·7

Comparison of Series A and B, Table I, shows that the effect of dilution on the stale medium, whether it be with apple extract or with water, is to restore the capacity for germination and growth. In neither case is there any germination in stale extract diluted to one-half; beyond this point increasing dilution, within limits, results in increased growth.

When water is used as the diluent we find good growth in 1 per cent. staled extract compared with that in pure water, indicating that inhibition of germination in the staled medium is not due to lack of nutrient materials.

Effect of (a) *boiling*, (b) *restoration of pH*, (c) *both.* The following table shows the change in reaction of Richards' solution brought about by the growth of *Fusarium* sp., and also the effect of boiling the staled medium in restoring its germinative capacity for spores of *Botrytis cinerea.*

TABLE II.

Growth in control, 12·0. pH = 5·35.

	R.S.F.		*R.S.F. boiled.*	
	Average length of germ-tube.	*pH.*	*Average length of germ-tube.*	*pH.*
2 days stale	8·1	5·5	11·0	5·5
4 " "	6·3	5·6	8·8	5·6
6 " "	3·1	5·7	4·7	5·9
8 " "	0·0	6·6	4·7	6·4
10 " "	0·0	7·3	2·3	7·4

When *Fusarium* sp. is grown in Richards' solution the active acidity of the medium decreases with the growth of the fungus. The boiling of the staled medium results in increased growth of *Botrytis* spores compared with that in the unboiled, but the effect decreases as the medium becomes increasingly stale. The increased growth in the boiled liquid indicates the destruction of thermolabile substances which exerted a toxic influence on the staled medium.

TABLE III.

	Staled.		*Staled and boiled.*		*Staled + HCl.*		*Staled and boiled + HCl.*		*Weight of mycelium.*
	Bot. growth.	*pH.*	*Bot. growth.*	*pH.*	*Bot. growth.*	*pH.*	*Bot. growth.*	*pH.*	*Grm.*
				Richards' Solution (0·5 % *Phos.*).					
4 days stale	7·8	4·9	9·4	4·9	—	—	—	—	—
6 ,, ,,	3·0	5·8	5·0	5·9	—	—	—	—	0·4860
7 ,, ,,	—	—	—	—	—	—	—	—	—
8 ,, ,,	0·33	6·3	6·4	6·3	3·7	4·85	—	—	0·6355
10 ,, ,,	0·0	7·3	—	7·5	1 5	3·9	7·8	3·7	0·8785
12 ,, ,,	0·0	7·65	1·1	8·1	0·0	4·1	9·0	4·1	0·9145
14 ,, ,,	0·0	8·1	0·07	9·5	0·0	3·6	5·8	4·8	0·9840
				Richards' Solution (0·05 % *Phos.*).					
4 days stale	10·9	4·4	11·7	4·8	—	—	—	—	—
6 ,, ,,	1·6	4·4	7·2	4·4	—	—	—	—	0·1116
7 ,, ,,	—	—	—	—	—	—	—	—	—
8 ,, ,,	0·48	7·0	—	—	4·0	4·5	—	—	0·4570
10 ,, ,,	0·0	7·1	6·6	6·9	3·6	3·6	11·1	4·0	0·5965
12 ,, ,,	0·0	8·1	2·7	—	1·5	4·0	9·3	3·7	0·7260
14 ,, ,,	0 0	9·0	0·6	9·9	1·9	3·6	9·5	4·3	0·8425
				Potato Extract.					
4 days stale	0	8·2	4·7	8·9	—	—	—	—	—
6 ,, ,,	0	9·1	0·0	10+	—	—	—	—	0·1230
7 ,, ,,	0	8·5	0·0	10+	0	7·1	2·7	'acid'	—
8 ,, ,,	0	8·8	0·0	10+	0	7·5	0·0	7·0	0·1148
10 ,, ,,	0	8·9	0·0	10+	—	—	0·0	8·0	0·1310
12 ,, ,,	0	9·0	0·0	10+	0	7·3	0.0	7·2	0·1100
14 ,, ,,	0	9·0	0·0	10+	0	6·7	0·0	7·1	0·1335

As the reaction of the staled liquid approaches true neutrality the phosphates present are precipitated on boiling, and thus factors are introduced other than the destruction of any thermolabile substances which may be present. An attempt was made to get over this difficulty by reducing the amount of phosphate in normal Richards' solution (0·5 per cent.) to 0·05 per cent. while keeping the other constituents at the same concentration as before. The progress of staling in this medium as well as in normal Richards' solution and in potato extract is shown in the table above, also the results of experiments to restore the germinative capacity to the staled media. When the hydrogen-ion concentration was altered considerably by the growth of *Fusarium* sp., it was restored approximately to that of the unstaled controls by the addition of HCl $\frac{\text{N}}{1}$ to the staled solutions. The combined effect of boiling and the restoration of the pH value is also shown. All the flasks were inoculated with equal quantities of the same spore suspension and incubated at 20° C., uninoculated controls being kept under similar conditions. The growth in the controls is reckoned as 12·0 units in each case.

The initial hydrogen-ion concentrations were as follows:

Richards' solution	0·5 per cent. phosphate,	pH 4·25.
,, ,,	0·05 ,, ,,	pH 4·55.
Potato extract,	—	pH 7·0.

The comparative growth of *Fusarium* sp. on these three media, and also the progress of staling as indicated by the growth of *Botrytis cinerea* in the staled media, are graphically represented in Figs. 1 and 2 respectively. Comparison of the weights of mycelium produced on Richards' solution 0·5 per cent. phosphate, Richards' solution 0·05 per cent. phosphate, and potato extract shows that as media for the growth of *Fusarium* sp. they stand in the order named. The rates of growth of *Fusarium* sp. on potato extract and on Richards' solution containing the smaller amount of phosphate are very similar during the first six days, while that on normal Richards' solution is well in advance. While there is little or no further

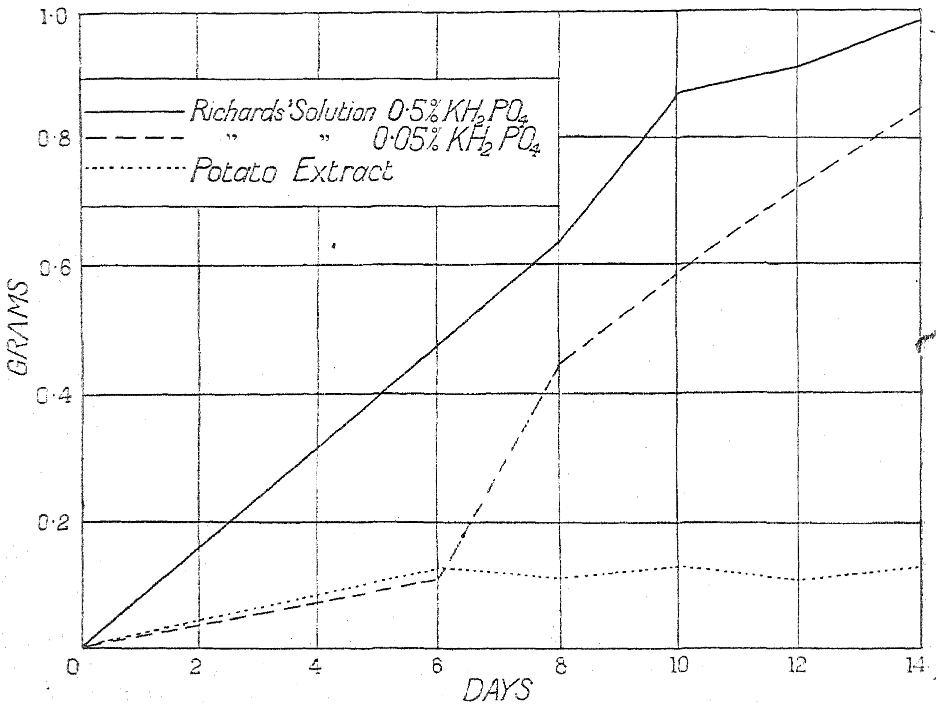

FIG. 1. Graphical representation of the comparative growth of *Fusarium* sp. on Richards' solution 0·5 per cent. KH_2PO_4; Richards' solution 0·05 per cent. KH_2PO_4; and potato extract.

development of mycelium on potato extract, the amount of growth on Richards' solution 0·05 per cent. phosphate increases steadily and closely approximates to that on normal Richards' solution at the end of fourteen days. The striking feature which the table shows, and which is graphically represented in Fig. 2, is the rapidity with which potato extract becomes staled in comparison with Richards' solution, notwithstanding the poor mycelial development on the former. At the end of four days the accumulation of toxic substances by *Fusarium* sp. growing on potato extract is sufficient to inhibit completely the germination of *Botrytis* spores when sown in the staled liquid. At this stage boiling restores its germinative and growth capacity to some extent. At a later stage, however, neither boiling nor restoration of the pH approximately to that of the control has any apparent effect in that direction. The cumulative effect of both, however, is sufficient to permit slight growth of *Botrytis cinerea* in a seven-day stale

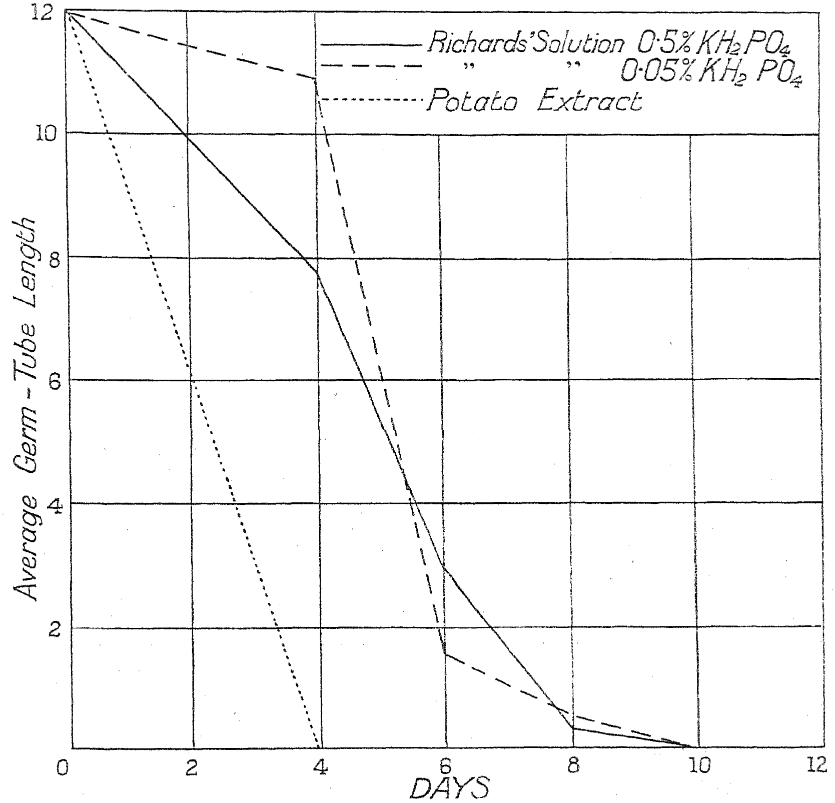

FIG. 2. Graphical representation of the comparative staling by *Fusarium* sp. on Richards' solution 0·5 per cent. KH_2PO_4; Richards' solution 0·05 per cent. KH_2PO_4; and potato extract, as indicated by the growth of *Botrytis cinerea* in the staled medium.

medium, but is without effect at a later stage. It should be noted that the seven-day stale medium was made 'acid' by the addition of HCl $\frac{N}{1}$: the exact hydrogen-ion concentration was not determined.

Comparison of Figs. 1 and 2 shows that the amount of staling on a particular medium is proportional to the development of *Fusarium* sp. The slight growth of *Fusarium* sp. on the four-day staled sample of Richards' solution 0·05 per cent. phosphate is accompanied by a small amount of staling. The eight-day stale sample shows considerably more growth and much greater staling properties.

The general features of staling on Richards' solution, normal and modified formulae, are very similar. In either case there is complete inhibition of germination of *Botrytis* spores in ten-day staled solutions and onwards. The inhibiting effect is partly removed by boiling and also by correction of the pH value. When the medium has reached that degree of staleness that the germination of *Botrytis* spores is just inhibited, the combined effect of boiling and correction of pH value is to restore to the staled medium almost the germination and growth capacity of the original. The effect of such treatment, however, decreases as the accumulation of toxic substances increases.

In Table IV the staling processes by *Fusarium* sp. in Richards' solution and apple extract are compared and followed to a somewhat later stage. All the flasks were inoculated with equal quantities of the same spore suspension and incubated at 20° C., uninoculated controls being kept under similar conditions.

The growth in the controls is reckoned as 12·0 divisions on the ocular micrometer. The initial hydrogen-ion concentrations were as follows: R.S., pH 5·35; A.E., pH 4·6.

TABLE IV.

	Richards' Solution.							*Apple Extract.*				
	Staled.		*Staled and boiled.*		*Staled, boiled, and HCl added.*		*Weight of mycelium.*	*Staled.*		*Staled and boiled.*		*Weight of mycelium.*
Days stale.	*Bot. growth.*	*pH.*	*Bot. growth.*	*pH.*	*Bot. growth.*	*pH.*	*Grm.*	*Bot. growth.*	*pH.*	*Bot. growth.*	*pH.*	*Grm.*
4	9·3	5·6	10·6	5·6	—	—	0·2450	3·6	5·1	4·0	5·1	0·0010
6	—	6·6	9·7	—	9·6	3·0	0·7505	1·3	5·2	1·9	5·2	0·0210
9	0·0	6·8	2·2	—	5·0	3·2	0·7520	—	—	—	—	0·0276
11	0·0	8·4	—	9·8	6·1	3·4	1·0042	1·5	5·3	5·3	5·3	0·0152
16	0·0	8·2	—	10+	3·6	5·8	0·9110	0·0	5·3	0·0	5·3	—
20	0·0	8·3	0·0	—	2·2	3·2	1·1036	0·0	5·3	0·0	5·3	0·0242
25	—	—	—	—	—	—	—	0·0	5·2	0·0	5·2	—

Comparison of the mycelial growth on Richards' solution and apple extract shows that the latter is a comparatively unsuitable medium for the growth of *Fusarium* sp. In contrast to Richards' solution, which shows a marked change in hydrogen-ion concentration towards the alkaline side with the growth of *Fusarium* sp., the change of reaction in apple extract, although in the same direction, is comparatively slight; consequently re-adjustment of the pH value of staled apple extract was not considered necessary.

The progress of staling is also seen to be much slower in apple extract, the stage of complete inhibition of germination of *Botrytis* spores only being reached in sixteen-day-old cultures, whereas the same stage is reached in six days in the case of Richards' solution.

The effect of boiling on the staled medium is similar in both cases, i. e. in the early stages the inhibiting factors are partly destroyed, but the effect disappears as the accumulation of toxic substances increases.

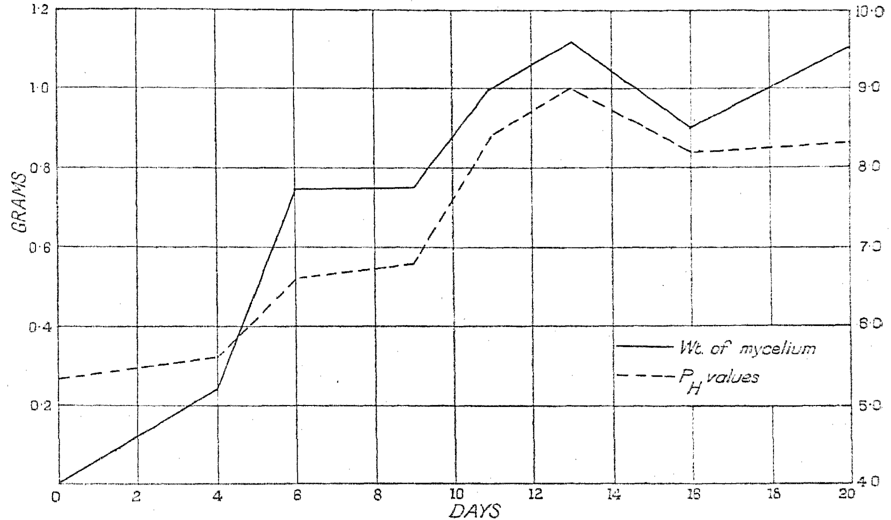

FIG. 3. Graph showing relation between rate of growth of *Fusarium* sp. on Richards' solution and rate of change of hydrogen-ion concentration of medium.

As was shown in Table III, boiling and correction of pH value of staled Richards' solution have a combined effect in promoting germination and growth of spores of *Botrytis cinerea*, even at a stage when they are separately ineffective.

It will be noticed that considerable variation in growth may occur in individual cultures of the same medium, even when every precaution is taken in selecting uniform flasks and inoculating equal quantities of medium with equal amounts of the same spore suspension. As a general rule the degree of staling and change in reaction of the medium is proportional to the amount of mycelial development irrespective of the age of the culture. The rate of change in reaction of the medium and the rate of growth of *Fusarium* sp. on Richards' solution are represented graphically in Fig. 3.

When grown on the three standard media used in the present investigation it has been shown that *Fusarium* sp. causes a definite decrease in hydrogen-ion concentration. When the pH value of the staled medium is restored approximately to that of the control, part at least of the toxicity is removed. Hence the question arises, to what extent is the decrease in hydrogen-ion concentretion responsible for the inhibition of growth in staled media? Working on the germination of spores of certain fungi in relation to hydrogen-ion concentration, Webb (8) found that the limit of germination on the alkaline side for spores of *Botrytis cinerea* in $\frac{M}{5}$ mannite at 22° C. was pH 7·0.

Reference to Table III will show that germination of spores of *Botrytis cinerea* took place in fourteen-day staled and boiled Richards' solution 0·05 per cent. phosphate having a pH value of 9·9. It would thus appear either that the strain of *Botrytis cinerea* used in these experiments has a much wider limit of pH values within which it can grow than that used by Webb, or that the limiting pH for germination is a function of the medium. To decide this question, various pH standards of Richards' solution, potato extract, and apple extract were prepared by the addition of varying quantities of HCl $\frac{N}{1}$ or NaOH $\frac{N}{1}$ to definite amounts of the different media. The standards were first approximately fixed by the addition of the requisite amounts of HCl $\frac{N}{1}$ or NaOH $\frac{N}{1}$ to 50 c.c. quantities of the different media. The flasks were then sterilized at 120° C. for twenty minutes and the pH values accurately determined afterwards. It was found that there was little or no change in those standards on the acid side of true neutrality as a result of autoclaving, while those at the alkaline end had altered considerably towards the acid side. These were further readjusted to the desired pH by the addition of fresh NaOH $\frac{N}{1}$. Precipitates were thrown down in Richards' solution and potato extract in those standards to the alkaline side of the true neutral point. These were allowed to settle to the bottom of the flasks and the supernatant liquid withdrawn for inoculation. $\frac{M}{5}$ mannite as used by Webb was also included in the series for purposes of comparison. The standard at the extreme alkaline end of the range in each case was beyond the limits of the indicator used and is designated 10+. The pH limits and optimum for growth of the *Fusarium* sp. used in the present investigation were also determined.

Drop cultures inoculated with the spores of the appropriate fungus were set up as described in the earlier part of the paper and incubated at 22·5° C. The results are shown in the following tables, and are graphically represented in Figs. 4 and 5. The curves are developed from the measurements taken after ten to twelve hours' growth.

TABLE V.

Growth of *Fusarium* sp. in Richards' Solution of various Hydrogen-ion Concentrations.

pH.	1·7	2·3	3·1	3·9	5·5	6·7	7·4	8·7	9·3	10+
6 hrs. growth	0·0	0·0	0·0	0·77	1·5	1·6	2·1	2·7	3·4	1·2
11 hrs. growth	0·0	0·0	0·63	4·7	6·0	6·3	8·4	10·5	12·0	5·1

TABLE VI.

Growth of *Botrytis cinerea* in Richards' Solution of various Hydrogen-ion Concentrations.

pH.	1·7	2·5	3·1	3·9	5·5	6·7	7·4	8·7	9·5	10+
6 hrs. growth	0·0	0·91	1·9	2·4	2·6	2·7	1·1	0·37	0·1	0·0
11 hrs. growth	0·2	2·6	5·0	5·8	6·2	7·8	3·6	2·3	1·0	0·0

TABLE VII.

Growth of *Fusarium* sp. in Apple Extract of various Hydrogen-ion Concentrations.

pH.	1·4	2·2	3·4	4·3	5·0	5·9	7·2	8·4	9·5	10+
12 hrs. growth	0·0	0·0	0·76	3·0	3·0	4·7	6·3	7·3	7·5	6·0

TABLE VIII.

Growth of *Botrytis cinerea* in Apple Extract of various Hydrogen-ion Concentrations.

pH.	1·4	2·2	3·4	4·3	5·0	5·9	7·2	8·4	9·5	10+
11 hrs. growth	0·0	1·4	6·6	8·4	8·6	9·7	11·6	11·0	6·6	2·1

TABLE IX.

Growth of *Fusarium* sp. in Potato Extract of various Hydrogen-ion Concentrations.

pH.	1·5	2·2	3·3	4·2	5·3	6·5	7·1	8·5	9·3	10+
6 hrs. growth	0·0	0·0	0·18	0·56	1·6	1·6	1·7	1·7	1·6	1·8
12 hrs. growth	0·0	1·5	6·4	9·9	14·7	16·1	17·2	14·3	14·3	14·2

TABLE X.

Growth of *Botrytis cinerea* in Potato Extract of various Hydrogen-ion Concentrations.

pH.	1·5	2·2	3·3	4·2	5·3	6·5	7·1	8·5	9·3	10+
6 hrs. growth	0·0	0·65	1·6	1·6	3·1	2·8	2·5	1·8	0·73	0·46
10 hrs. growth	0·0	1·8	5·2	6·0	6·4	6·4	7·2	4·8	2·1	1·3

TABLE XI.

Growth of *Fusarium* sp. in $\frac{M}{5}$ Mannite of various Hydrogen-ion Concentrations.

pH.	1·9	3·0	4·2	5·4	6·8	7·9	8·5	9·2	10+
12 hrs. growth	0	0	3·4	8·6	8·3	8·1	7·6	6·7	2·3
Percentage germination	0	0	96	100	100	100	100	97	89

TABLE XII.

Growth of *Botrytis cinerea* in $\frac{M}{5}$ Mannite of various Hydrogen-ion Concentrations.

pH.	1·9	3·0	4·2	5·4	6·8	7·9	8·5	9·2	10+
11 hrs. growth	0·67	3·3	9·9	11·3	5·2	0	0	0	0
Percentage germination	90	97	100	100	98	0	0	0	0

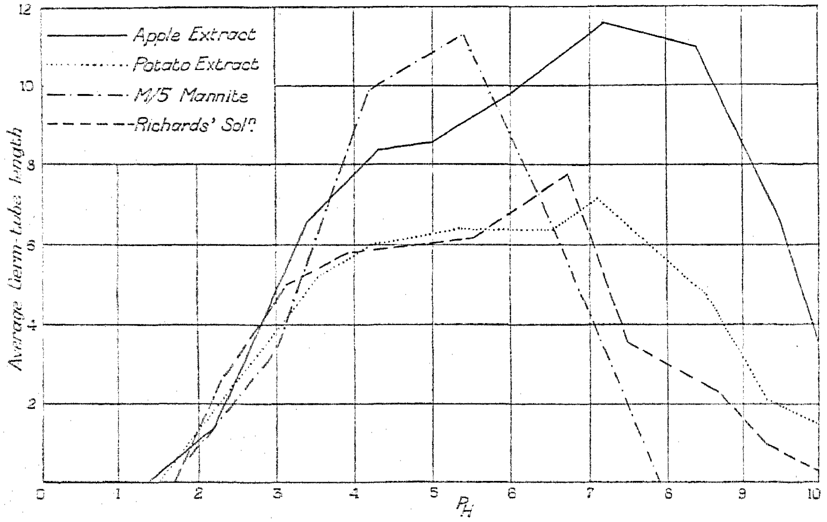

FIG. 4. Graphical representation of the growth of *Botrytis cinerea* on Richards' solution, potato extract, apple extract, and $\frac{M}{5}$ mannite of different hydrogen-ion concentrations.

Reference to Tables V to XII and Figs. 4 and 5 shows that the pH limits and optimum for growth of the two fungi used are not absolute in either case, but depend on the medium on which the fungi are grown.

In the case of *Botrytis cinerea* grown on Richards' solution, potato extract, and apple extract, the optimum growth was obtained round about the point of true neutrality. On $\frac{M}{5}$ mannite, Fig. 4 shows that the crest of the curve is well on the acid side, the optimum growth being obtained in the medium having a hydrogen-ion concentration of pH 5·4. Beyond this point there is a rapid diminution of growth with decrease in hydrogen-ion concentration, germination being totally inhibited in cultures having a pH exponent 7·9 and higher. The pH limits for germination of spores of *Botrytis cinerea* in $\frac{M}{5}$ mannite shown in Table XII are in accord with the results obtained by Webb. At 22° C. he got 46 per cent. germination at pH 7·0, and no germination at pH 7·4. Table XII shows that the limit on the alkaline side lies between pH 6·8 and pH 7·9. The maximum germination which he obtained was 90·2 per cent., which occurred at pH 3·1. In Tables XI and XII the percentages of germination obtained in the different pH standards of $\frac{M}{5}$ mannite are recorded, and will show that such test would be of little value as a basis of comparison in the present case. The germination percentages obtained by Webb appear to be unusually low, even for a medium containing mannite as the sole nutrient.

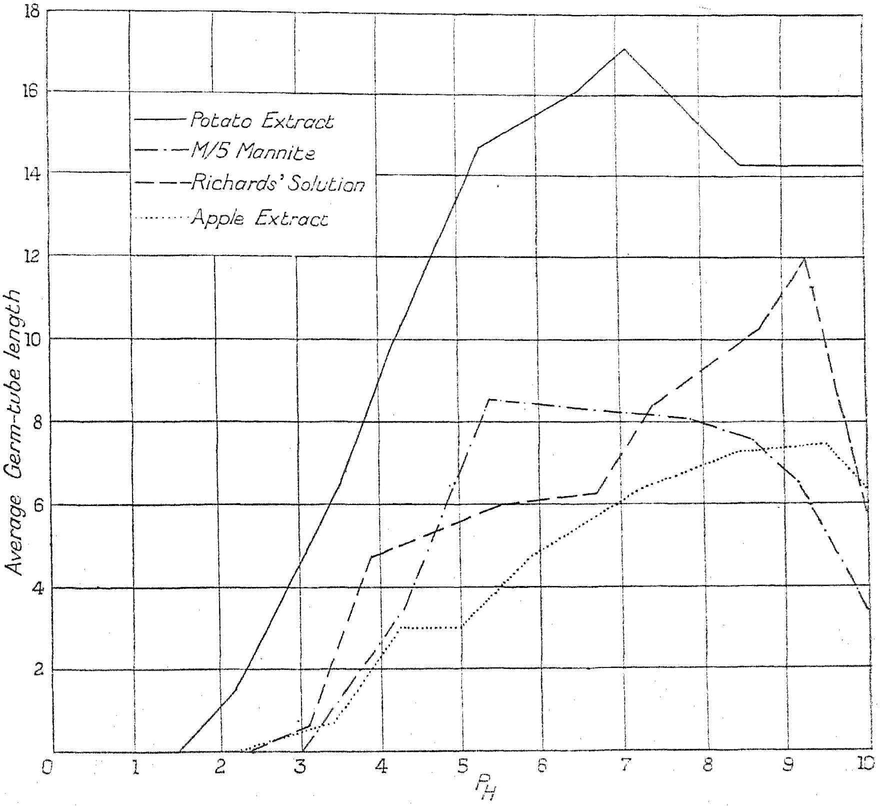

FIG. 5. Graphical representation of the growth of *Fusarium* sp. on Richards' solution, potato extract, apple extract, and $\frac{M}{5}$ mannite of different hydrogen-ion concentrations.

The pH limits and optimum for growth of *Fusarium* sp. vary according to the media used. Potato extract gives germination within the limits pH 2·2 and pH 10+ with maximum growth about the neutral point. In this medium *Fusarium* sp. appears to be very tolerant of a relatively high concentration of OH ions, giving comparatively good growth in cultures of pH greater than 10. In Richards' solution and apple extract maximum growth is obtained in the alkaline end of the range, viz. at pH 9·3 for the former and pH 9·5 for the latter. The pH limits of growth on these two media are very similar, germination being obtained within the limits pH 3·4 and pH 10+ in both. When grown on mannite, *Fusarium* sp. showed no very pronounced maximum, but gave good growth within the limits pH 5·4 and pH 8·5. There was no indication of a secondary maximum as observed by Webb.

In the media used *Fusarium* sp. appears to be more tolerant of OH ions than *Botrytis cinerea*, while the latter can germinate at higher hydrogen-ion concentrations than can the former. In general, OH ions appear to have a greater inhibiting effect on the growth of both fungi than H ions. Peculiar formative effects were noticed in cultures which were obviously unsuitable for germination by reason of their high hydrogen-ion concentration. For example, spores of *Botrytis cinerea* in apple extract of pH 2·3 appear to bud like a yeast, while spores of *Fusarium* sp. in cultures of Richards' solution of pH 2·3 produce misshapen germ-tubes after twenty-four hours such as are never observed in a normal nutrient medium.

Precipitation with alcohol. It has already been established that the germinative capacity can be partially restored to a staled medium by boiling. This suggests the presence of something of enzymatic or volatile nature. Preliminary attempts have been made to isolate some of the staling products by applying to the staled medium the technique of enzyme precipitation, the method being as follows: To 20 c.c. of the staled liquid were added 80 c.c. of absolute alcohol. The precipitate formed was filtered off and the filtrate evaporated to dryness. The residue left on the filter-paper was dried so as to get rid of the last traces of alcohol. Both residues were taken up in 10 c.c. distilled water. Equal proportions of each of these solutions were reunited again and the germinative capacity of solutions of the residues separately, and united, tested by the usual method. It should be pointed out that it is at present impossible to say what proportion of the nutrients present is precipitated by the alcoholic treatment. The taking up of both residues in 10 c.c. quantities of water is purely arbitrary and may in no way represent the concentration of nutrients in the medium. Controls in which the unstaled medium was treated in exactly the same manner were carried out at the same time. The results shown in Table XIII are typical of those obtained by such treatment. In the table, 'Staled res.' refers to the solution obtained by the addition of 10 c.c. of water to the precipitate formed by the addition of 80 c.c. absolute alcohol to 20 c.c. staled medium. 'Staled fil.' denotes the solution obtained by the addition of 10 c.c. water to the residue left after evaporation of the alcoholic filtrate to dryness. 'Staled res. + fil.' indicates equal quantities of both solutions mixed together.

TABLE XIII.

	Staled.	*Staled res.*	*Staled fil.*	*Staled res. + fil.*
	Bot. growth.	*Bot. growth.*	*Bot. growth.*	*Bot. growth.*
R.S.F. 8	0·0	5·2	1·6	2·1
R.S.F. 14	0·0	4·5	0·0	0·0
R.S.F. 16	0·0	1·9	0·0	0·0
R.S.F. 20	0·0	0·0	0·0	0·0

The effect of the alcohol treatment on Richards' solution is shown in the following table. The result quoted is the average of four separate precipitations.

TABLE XIV.

	R.S.	*R.S. res.*	*R.S. fil.*	*R.S. res. + fil.*
Botrytis growth	12·0	15·9	4·4	12·0

The same treatment has been applied to staled and unstaled potato and apple extracts with parallel results. In the early stages of the staling process, treatment with alcohol deactivates some of the growth-inhibiting factors. The effect becomes less apparent as the medium becomes increasingly staled. The results obtained from similar treatment of unstaled Richards' solution help to explain the comparatively good germination obtained in the staled residue. Treatment with alcohol precipitates most of the nutrients present in Richards' solution. The taking up of the precipitate and the residue from the alcoholic filtrate each in 10 c.c. of water results in a greater concentration of nutrients in the former and less in the latter than that present in the original medium. Hence the greater growth in 'R.S. res.' and smaller growth in 'R.S. fil.' than in normal Richards' solution, while the reconstituted 'R.S. res. + fil.' gives similar growth to the control.

The reconstituted staled medium gave a liquid which was less staled than the original. It is not at present clear where the deactivation of toxic substances takes place. The fact that the 'Staled res.' gave greater growth than the 'Staled fil.' does not necessarily indicate that the former contains less toxic substances than the latter, since the control experiments show that most of the nutrients are precipitated by alcohol.

Ether extraction. An attempt was made to extract soluble toxic organic substances which might be present by treating staled Richards' solution with ether, but only with negative results.

Oxalic acid. Tests for the presence of oxalates in the staled media gave negative results.

Filtration. The effect of filtration was tried by forcing a nine-day stale Richards' solution through a collodion membrane at a pressure of 50 cm. Hg. The membrane was prepared according to the method of Brown (2), and in the nomenclature of the author would be described as a 90 per cent. membrane. The filtrate was inoculated with spores of *Botrytis cinerea*, control cultures in staled and unstaled Richards' solution being set up at the same time, and incubated for twelve hours at 20° C. The results are shown in Table XV.

TABLE XV.

	R.S.	*R.S.F.*$_9$.	*R.S.F.*$_9$ *Filtrate.*
Botrytis growth	12·0	0	2·0

The result shows that some of the growth-retarding substances are removed from stale Richards' solution by passage through a collodion filter. It is quite possible that by using less permeable membranes still more of the toxic substances present may be removed from staled media.

GENERAL DISCUSSION.

The three standard media used in the present investigation are found to give characteristic types of staling when sown with spores of *Fusarium* sp. On Richards' solution the fungus grows luxuriantly and stales the medium comparatively slowly. Potato extract is a less favourable medium for mycelial development, but the staling process here is extremely rapid, the stage of complete inhibition of germination of spores of *Botrytis cinerea* being reached, under the conditions of the experiment, in three or four days. Apple extract is a very unfavourable medium for the growth of *Fusarium* sp., the fungus never getting beyond the stage of forming a thin covering of mycelium over the culture medium. The staling process is correspondingly slow, but is exceptional compared with that in Richards' solution, in that complete staling is reached with slight change in reaction of the medium.

On all three media the fungus causes a decrease in hydrogen-ion concentration. It is difficult to specify to what extent this change is responsible for the toxicity of staled liquids. The restoration of its germinative capacity to a slightly staled medium by readjustment of its pH value approximately to that of the original would indicate that the latter is a limiting factor in such cases. At a later stage in the process, however, correction of the pH value has no apparent effect in promoting germination and growth, doubtless due to the accumulation of other inhibiting factors. The experiments on the growth of *Fusarium* sp. and *Botrytis cinerea* in various hydrogen-ion concentrations of different media show that the pH limits of growth depend both on the fungus and on the medium. Since the medium is continually changing during the staling process the pH limits of growth may alter in consequence. For this reason the influence of the change in reaction is difficult to determine, but in general it appears to be concomitant with the staling process and within wide limits is not, in all probability, a limiting factor in germination and growth. The effect of boiling in promoting growth in slightly staled media shows that some of the inhibiting factors are of a thermolabile nature. The accumulation of thermostable toxic substances is sufficient to obliterate the effect of boiling at a later stage.

The nature of the thermolabile toxic substances is at present not clear. Previous work has shown that the production of NH_3 by some fungi has a retarding influence on their growth and development, the amount of NH_3 produced depending on the medium. Although the presence of NH_3 could be demonstrated in the staled solutions in the present case, the quantity did not appear to be sufficiently in excess of that present in the controls to account for the thermolabile toxicity of staled solutions.

The results obtained from the alcoholic precipitation of staled media are difficult to interpret at the present stage. The important point which is clearly established is that in the early stages of staling alcoholic treatment deactivates some of the inhibiting factors.

Table XV shows that filtration through a collodion membrane removes some of the injurious factors from stale Richards' solution, indicating that some of the toxic substances present in stale Richards' solution are colloidal in nature.

SUMMARY.

When grown for a time on Richards' solution, potato extract, or apple extract, *Fusarium* sp. renders the medium unsuitable for further growth.

The toxic action of the staled media is not specific; spores of *Botrytis cinerea* are more susceptible to the metabolic products of *Fusarium* sp. than are the spores of *Fusarium* itself.

The criterion of the progress of staling has been the degree of inhibition of germination and growth of *Botrytis* spores when sown in the staled media.

Fusarium sp. grows luxuriantly on normal Richards' solution, the staling process being gradual. The reaction of the medium becomes alkaline, the pH value changing from 4·0 to 8·0 in about two weeks under the conditions of the experiment.

Growth on Richards' solution containing 0·05 per cent. KH_2PO_4 proceeds slowly at first, but increases steadily, and at the end of two weeks closely approximates to that on normal Richards' solution. The reaction of the medium changes from about pH 4·5 to pH 9·0 in two weeks.

Potato extract is a less favourable medium for the growth of *Fusarium* sp., the maximum development being reached in about six days. The extract stales very rapidly. The reaction of the medium quickly becomes alkaline, the pH value changing from 7·0 to 9·1 in six days.

The growth of *Fusarium* sp. on apple extract is very meagre and the staling slow. The active acidity of the medium is reduced from pH 4·6 to pH 5·3 in eleven days, after which there is no further change in hydrogen-ion concentration.

The retardation of growth on staled media is not due to lack of nutrients.

In the early stages of staling the inhibiting action of a staled medium on germination and growth can be partly removed by boiling.

When the hydrogen-ion concentration of the medium has been altered considerably by the growth of the fungus, the growth-retarding effect can be partly removed by restoring the pH value of the staled medium approximately to that of the control.

Boiling and correction of the pH value have a cumulative effect in promoting growth in a staled solution when separately they may be ineffective.

The pH limits and optimum for growth of *Fusarium* sp. and *Botrytis cinerea* depend on the medium.

Within wide limits the pH value of a staled solution does not appear to be a limiting factor in growth.

Filtration through a 90 per cent. collodion membrane removes some of the toxic properties from a staled medium.

The toxic substances in a staled solution are partly deactivated by precipitation with alcohol; the reconstructed medium is less 'stale' than the original.

The author wishes to express his indebtedness to Professor V. H. Blackman, who suggested the present investigation, and to Dr. W. Brown for his continuous interest and valuable assistance throughout its prosecution.

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The Physiology of Fusarium coeruleum.

BY

ENID S. MOORE, PH.D.

With Plates VII and VIII and ten Figures in the Text.

UNTIL recent years, rots of the potato due to species of *Fusarium* have been the subject of much confusion and dispute. The old name *F. solani* was shown by Appel and Wollenweber (1) to include several distinct species, distinguishable both on morphological grounds and by their behaviour as parasites. Wollenweber's (23) systematic subdivision of the genus emphasized the distinction between species which produce only a rot of the tubers and those which may also cause wilt by attacking the vascular system. Both Carpenter (5) and Link (13) have contributed towards the establishment of a morphological and physiological distinction, whilst Sherbakoff (21) enumerates no less than sixty-one distinct species and varieties of *Fusarium* isolated from rotted potatoes.

Amongst the authors already named, and others quoted later, is to be found some account of the morphology and cultural characters of *Fusarium coeruleum*, (Lib) Sacc.; the only comprehensive description of the organism is, however, that of Pethybridge and Lafferty (18), who deal with the general characters of the fungus on vegetable media and with its behaviour as a wound-parasite. They establish beyond doubt that it is the true cause of the dry-rot of potatoes in storage, where it is responsible for considerable losses, especially in the early months of the year.

The present investigation was undertaken with particular reference to the physiology and parasitism of the organism.

EXPERIMENTAL METHODS.

Two strains of *Fusarium coeruleum* have been used in the course of the investigation. One (C) was obtained from the Central Bureau at Amsterdam, where it was originally identified by Wollenweber. Later experiments were carried out with a culture (4) isolated in March 1922

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from a potato showing characteristic dry-rot. In both cases the starting-point was a culture originating from a single spore. The second strain, whilst typical both in morphology and in pathogenicity, differed from the Amsterdam culture in several points. It had a much stronger capacity for colour-production, and comparative trials showed it to be a much more vigorous parasite. It differed also in the readiness with which it produced conidia. Strain C always tended to develop chlamydospores rather than macroconidia, and the latter did not usually appear until the cultures had reached a considerable age. With the new strain, on the other hand, macroconidia were formed readily in young cultures, and appeared in abundance on many media, both scattered over the mycelium and aggregated in buff-coloured or blue-green spore-masses.

Vegetable media were used in the first instance for the preliminary study of the organism. On potato agar (thick) a vigorous, white, erect mycelium is developed, and the characteristic indigo-blue colour appears in the substratum. The same colour arises in culture on cooked potato plugs and also frequently on inoculated raw tubers. On a more dilute medium (potato-extract agar) no colour appears, and the erect aerial growth tends to be replaced by a weft of horizontal hyphae forming a smooth surface film. On Quaker-oat agar, brilliant purple appears in the substratum underlying a vigorous aerial mycelium. On oat-extract agar both colour and the erect habit are suppressed. On cooked rice a red-purple is developed. On the weaker media macroconidia were formed earlier, but not abundantly, whilst chlamydospores frequently developed in great numbers.

These early preliminary experiments emphasize the great variation shown by the fungus, not only in the type and relative abundance of reproductive organs, but also in the development of colour and in the habit of growth. Sherbakoff (l. c.) in fact remarks that 'it might appear at first glance that the variation of these fungi is so great as to leave no firm ground for morphological treatment of the group'. His own examination of forms of *Fusarium* was carried out on vegetable media, such as potato agar, potato, rye straw, oat grains, &c.

It was thought that light might be thrown on the variations noted above and their relation to the environment if a closer study were made of growth on synthetic media. Several media have been tried, but eventually the formula devised by Coons (7)[1] was taken as a basis and modified in various ways as the experiment demanded. Unless otherwise stated, the media were in all cases adjusted to definite H-ion concentration (pH 6·5 approximately). Sterilization was usually effected by steaming for three successive days, although media were occasionally autoclaved at 120° C.

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[1] Coons' medium, normal strength, is KH_2PO_4 M/100, $MgSO_4$ M/500, maltose M/100, asparagin M/100. In the experiments described the two first constituents are indicated by *S*.

The examination involved:

1. Petri-dish cultures of the fungus inoculated at a central point on media containing 2 per cent. agar. The diameter of the colony was determined by measurements in two directions, at least two parallel cultures being used for each trial.

2. Flask cultures of the fungus in liquid media, for the determination of dry weight of mycelium formed. Change in reaction of the medium was examined in these cultures also, by means of one or other of the colorimetric methods described by Clark (6). At least two similar cultures were inoculated for each test, and when possible a larger number was used.

3. Measurement of the rate of germination of spores in liquid media, using the technique described by Brown (3).

For inoculation a water-suspension of spores (preferably unaccompanied by mycelium) was introduced by means of a sterile loop or pipette. Precautions were taken to ensure uniformity of inoculation and of controllable external conditions throughout each series of cultures.

Influence of Temperature.

Some of the temperature-relations of *F. coeruleum* have been examined by Edson and Shapovalov (8), who adopted as their standard of measurement the diameter of the fungal colony upon potato agar. They concluded that the growth optimum lies at about 25° C., while the minimum and maximum lie at 5° C. and 30° C. respectively. At the latter temperature the spores do not germinate.

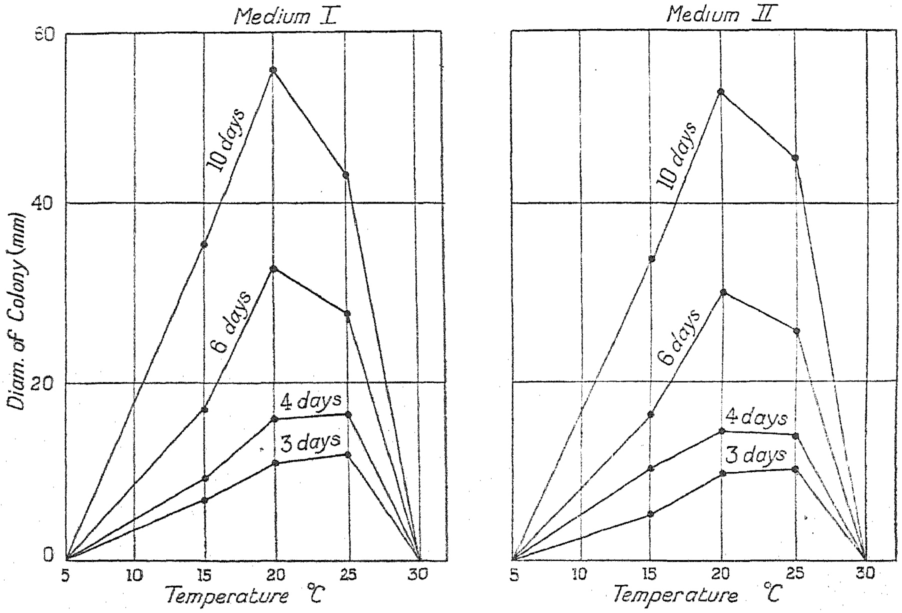
These conclusions have not been fully confirmed in the present investigation by the three methods outlined above. Preliminary experiments on potato agar indicated that the rate of expansion is greater at 20° C. than at 25° C., while growth, although slow, is still possible at 30° C. In another representative trial a synthetic medium was selected, consisting of S + KNO_3 M/50 + sucrose M/20. This was used both at the concentration given (medium I) and also at half the total concentration (medium II). In both cases 2 per cent. agar was added. The following figures represent the average obtained from five similar cultures (C):

Growth on Solid Media at Different Temperatures.

	Medium I.					*Medium II.*				
Incubation Temperature, ° C.	5°	15°	20°	25°	30°	5°	15°	20°	25°	30°
Diam. (mm.) after 3 days.	0	6·7	11·0	12·0	0	0	5·2	10·0	10·6	0
,, ,, 4 ,,	0	9·2	16·3	16·7	—	—	10·4	15·0	14·5	0
,, ,, 6 ,,	0	17·4	33·2	28·0	—	—	16·8	30·4	26·0	0
,, ,, 10 ,,	0	35·7	55·7	44·0	4·2	0	34·2	53·4	46·0	2.2

These measurements (represented graphically in Text-fig. 1) again indicate that on both media the temperature allowing maximum growth lies between 15° and 25° C., and probably between 20° C. and 25° C. A slow and stunted development occurs at 30° C. In another experiment it was found that during thirty-seven days extensive growth had taken place at 5° C., although none was apparent at the close of the ten-day period of an earlier trial.

Marked differences were noticed in the type of growth developed at different temperatures. At 5° C. the fungus produces a thin weft of vegetative hyphae, forming a film over the surface of the agar. At 15° C. a scanty and diffuse aerial mycelium is occasionally present, whilst the extensive surface film bears abundant conidia. At 20° C. the aerial growth is much more vigorous and compact, and the surface film is limited to the peripheral zone of the colony. This film is still more restricted in area in the cultures incubated at 25° C., and is absent altogether at 30° C. At the latter temperature the beginning of growth is delayed and uncertain, and failed in ten out of the twenty cultures examined. In the cases where growth had started, the colony consisted of a small dense tuft of compact aerial mycelium, well raised above the agar, and with crinkled and crenate margin. A deep blue or purple colour developed gradually, while cultures at lower temperature remained colourless. The typical appearance of colonies on synthetic medium I is shown in Pl. VII, Fig. 1.



TEXT-FIG. 1. Graphs showing the rate of increase of colonies on two different media, at temperatures between 5° and 30° C.

A single determination was made of the dry weight of mycelium formed in seven days in the synthetic medium I. The figures given represent the total weight obtained in three flask cultures at each temperature (strain C).

Growth in Liquid Media at Different Temperatures.

	15° C.	25° C.	30° C.
Dry weight of mycelium (grm.)	0·116	0.247	0

It was repeatedly shown that no appreciable growth takes place in flask cultures at 30° C.

The Amsterdam strain, as already stated, presented difficulties with regard to spore-germination tests, and only one trial was therefore made with it. The experiment was repeated, using conidia from the newly-isolated strain (4), when the same synthetic medium (I) was compared with Coons' medium (normal concentration). The following measurements were obtained:

	° C.			
	15	20	25	30
Average length of germ-tube (7 hrs. germination):				
Coons' medium	0·9	4·3	7·7	1·3
Synthetic medium I	1·5	4·6	7·7	2·8

It appears that spores germinate more rapidly at 25° C. than at 20° C., and that under the conditions prevailing in an isolated drop of medium they germinate readily at 30° C., but do not continue a vigorous growth at that temperature.

The Thermal Death-point of F. coeruleum.

In 1909 Longman (14) examined the heat resistance of spores and mycelium of a fungus causing a dry-rot of potatoes, and described by her under the name '*Fusarium solani*'. It is doubtful whether her fungus is identical with *F. coeruleum*, or whether it is one of the wilt-producing type. She states that the conidia survive a temperature of 64–65° C. for ten hours if heated dry, but are killed at 50° C. when wet.

Preliminary experiments with conidia of *F. coeruleum* (C) showed that very few spores germinated after heating in water for twelve minutes at 44–46° C. In repeating the trials with younger conidia of strain 4, the following method was adopted: A suspension of spores in water was heated in a water-bath, samples being pipetted out at intervals and added to a weak nutrient solution. This was placed in drops on slides, and kept for a long period under conditions favourable for germination. The results are given below.

Effect of Heat (41–46° C.) *upon Germination Capacity.*

Temperature.	*Time heated*	*% germinated after*		
°C.	(min.).	22 hrs.	42 hrs.	66 hrs.
41	2	98		
	5	98		
	10	99		
	15	89	90 + ...	
	40	2	91	
43	2	96		
	5	95		
	10	47	92	
	17	3	89	
45	2	94		
	5	0	83	
	10	0	15	
	15	0	0	0
46	2	16	55	
	5	0	48	
	10	0	0	0·5
	15	0	0	0
Control	0	98		

These figures indicate that the treatment, whilst not actually killing the spore, may yet retard subsequent germination, to a greater or less extent, according to both the duration and intensity of the heat applied. A closer examination was made of the injury—expressed in terms of delayed germination—resulting from heating in water, at one temperature, for a period varying from two to ninety minutes. The temperature selected was 42·0–42·5° C., the spores being subsequently incubated in drops of nutrient medium at 15° C. The results given below show that heating at this temperature for over an hour is required to kill all the spores, whilst treatment for eight to ten minutes is sufficient to produce appreciable retardation of germination.

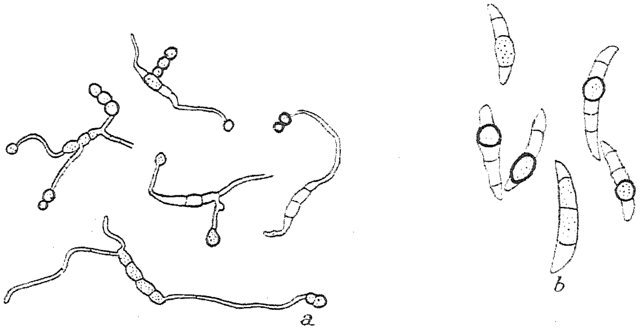
Effect of Heat (42·0–42·5° C.) *upon Germination Capacity.*

Time heated (min.).	*% germinated after*			
42·0–42·5° C.	16 hrs.	25 hrs.	40 hrs.	4½ days.
0	92			
2	97			
4	100			
8	89	97		
10	66	93		
12	54	94		
16	3	94		
20	10	62	88	
24	2	24	91	
28	0	13	82	
32	0	7	75	
41	0	0	28	
48	0	0	11	Many ungerminated.
60	0	0	4	Few germinated.
90	0	0	0	0

The effect is the same, whether the spores are heated in a dilute nutrient solution or in water. Dry heat produces slightly less injury in the longer periods of treatment.

It was noticed also that a prolonged exposure (twenty-two hours) to a temperature as low as 36° C. is sufficient to kill the conidia, whilst trial showed that none survive six days' treatment at 30–33° C., either submerged or dry.

These results are of interest in relation to the death-point of the fungus in an infected tuber. Inoculated tubers showing advanced rotting were cut into blocks measuring approximately 2 cm. in each direction, and placed in tubes of heated sterile water for varying periods. Subsequent incubation on agar showed whether the fungus was still living. It was found that while heating for thirty minutes was sufficient to kill the fungus at 45° C., certain death did not result from even four hours' treatment at 40° C. The fungus is therefore more resistant than *Phytophthora*, which, according to Jones, Giddings, and Lutman (12), is killed by the latter treatment, which, however, does not harm the germination capacity of the tuber.



TEXT-FIG. 2. (*a*) Conidia germinating in nutrient agar under reduced aeration, and showing the early formation of chlamydospores. (× 250.) (*b*) Conidia, under reduced aeration, becoming converted into chlamydospores. (× 425.)

Aeration.

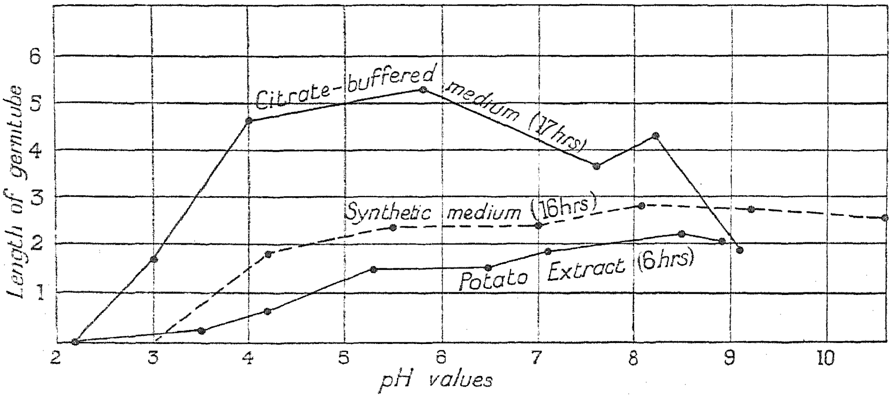
The effect of aeration has not been studied in detail, but certain isolated observations may be worth recording. It was noticed that chlamydospores were produced almost immediately by conidia germinating in nutrient agar under conditions of reduced aeration. Sometimes the spore appears at the end of a short germ tube, as a terminal swelling whose walls subsequently thicken. Frequently the conidium itself is converted to a chlamydospore, one or two of its cells becoming densely granular at the expense of the others, and developing the typical thick wall. The formation of such chlamydospores is shown in Text-fig. 2.

Colour-formation appears to be dependent on sufficient aeration, since in liquid cultures the blue colour arises first (and often exclusively) in the part of the mycelium exposed to air. The submerged weft remains colourless, and the contrast is very marked, especially in flask cultures containing a considerable depth of liquid.

Range of Acidity and Alkalinity tolerated by the Fungus.

It is stated (Pethybridge, 18, p. 208) that this fungus develops alkalinity as the result of its growth, whether on artificial media or on an infected tuber. He further states that the colour formed varies from red to blue, according to the acidity or alkalinity of the medium.

The exact range of reaction at which growth is possible has not hitherto been determined, and the following investigations were carried out with this object in view. Preliminary trials of spore germination were first made, using apple extract and potato extract, adjusted to definite pH values by the addition of NaOH or HCl. These were repeated, using a synthetic medium (S. + asparagin + sucrose). The data obtained in various experiments are shown graphically in Text-fig. 3, and indicate that conidia of *Fusarium coeruleum* can germinate over a very wide range. The maximum acidity tolerated lies a little below pH 3·0, whilst growth is still possible at pH 11·0.



TEXT-FIG. 3. Graph showing the length of germ tube developed in media at various H-ion concentrations.

It has already been pointed out that the initial reaction of the medium is altered by the metabolism of the fungus. Special methods must therefore be devised to study the continued growth in media of constant reaction. It was hoped to meet this difficulty by using a medium which, by addition of a suitable buffer-mixture, would maintain its original H-ion concentration unchanged during the progress of the culture. Gillespie (9), working with *Actinomyces chromogenus*, used either citric, tartaric, or succinic acid for this purpose, and, by subsequent adjustment with NaOH, obtained a medium which in the case of citrate suffered usually only a small change in exponent during thirty-seven or forty-eight days' culture.

Citric acid was selected, and was included (0·3 per cent.) in a medium containing S. + KNO_3 M/50 + sucrose M/10 or M/100. NaOH or HCl were added in varying amounts to produce the required reaction, and the exact pH value was determined, from a sample tube, after steaming on three successive days. This medium was used both in flask cultures and with the addition of 2 per cent. agar. In the former case the H-ion concentration of the medium was determined again at the conclusion of the experiment. Typical results are tabulated below.

Growth in Media containing Sodium Citrate.

	Sucrose M/10.			*Sucrose M/100.*			
Initial pH	7·4	5·8	3·9	8·2	6·0	4·3	3·0
Final pH (9 days)	6·2	5·3	4·2	9·2	9·2	9·0	3·0
Dry weight ,, (grm.)	0·057		0·088	0·218	0·208	0·233	0
Diam. (10 days) (mm.)	41·0	48·5	37·5	49·5	43·0	36·5	0

Two flasks of each liquid culture.
Two plates ,, ,, ,, .

It will be noticed that with sucrose M/10 the amount of mycelium formed was small, and the buffer action was maintained fairly well during the nine days of the experiment. In the second series, in spite of the lower concentration of sucrose (M/100), a larger amount of growth has taken place and the buffer action has ceased, a high degree of alkalinity being produced. These results, confirmed in other experiments, suggested that the citrate itself was being consumed, with liberation of free alkali from the base. That this was the case was proved by a quantitative comparison of the amount of growth in liquid media in which sucrose was replaced by potassium citrate as the sole carbon supply. The following results were obtained:

Medium = S. + KNO_3 M/50	Initial pH.	Final pH.	Dry weight (grm.).
+ sucrose M/50	6·4	5·6	0·951
+ ,, M/100	6·4	5·8	0·035
+ K citrate M/100	6·4	9·2	0·021
+ no carbon compounds	6·4	6·3	Negligible

The method, therefore, was of little service in elucidating the problem under consideration. Spore-germination trials gave results similar to those with potato and apple extract (see Text-fig. 3).

Influence of the Concentration of the Medium.

In early cultures on potato and oat agar, it appeared that the presence or absence of aerial mycelium was strongly influenced by the concentration of the medium. This point was further examined by trials upon a synthetic medium, diluted to various concentrations, both with and without agar. Coons' medium at normal strength (N) was selected as the basis of the series, and comparison was made with concentrations both higher and lower, viz. 10N, 2N, N/2, N/10.

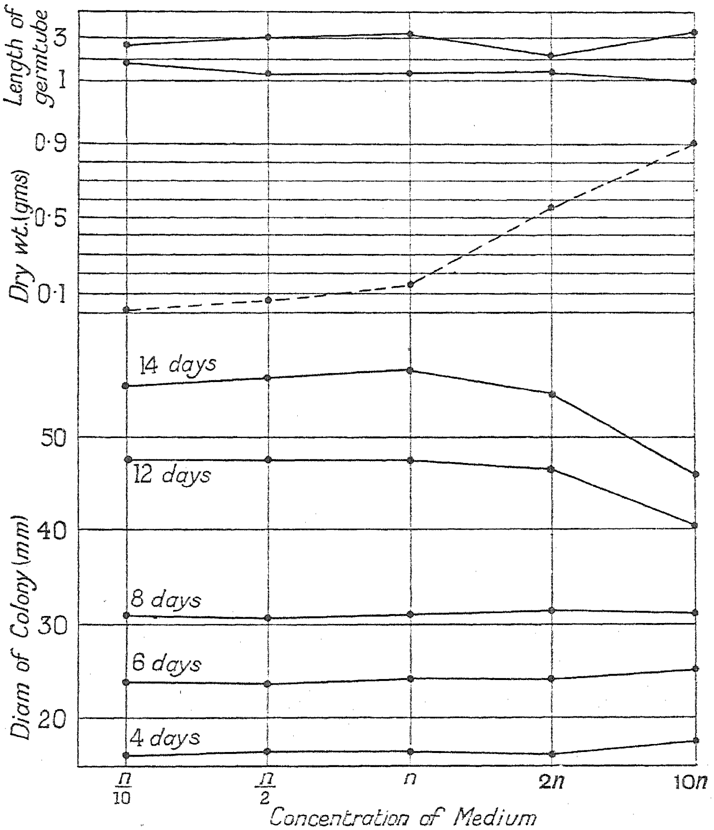
The difference in habit of the fungus on these various media (+agar) is very marked. At 10N concentration a vigorous aerial mycelium is developed, covering the whole surface of the colony. The mycelial weft is deeply crinkled, the corrugations being plainly visible from the under surface of the culture. It was noticed that, at this concentration, crystals (probably of asparagin) are deposited in tree-like growths in the solid medium. At 2N concentration the colony is smooth and level, the aerial mycelium being surrounded by a marginal zone where a surface film only is present. As the concentration is further lowered, the aerial growth becomes more and more reduced, both in area and in denseness, until at N/10 it is entirely suppressed even at the starting-point of the colony, only a thin and uniform film being produced. Five typical colonies are represented in Pl. VII, Fig. 2.

Corresponding differences appeared when the fungus was grown in liquid media in flasks. With high concentration there is developed a strongly crinkled weft of mycelium, of which a considerable part stands exposed above the liquid, and is there coloured blue or purple. On the more dilute media the weft is thinner, more or less entirely submerged, and colourless. At N/10 the weft is hardly even coherent. Measurements were made of colony diameter, dry weight, and of length of germ tube. The results are shown in the table below, and graphically in Text-fig. 4.

Growth of the Fungus on Media of various Concentrations.

	Concentration of Medium.				
	10N	2N	N	N/2	N/10
pH after 9 days	8·2	7·2	7·8	7·0	7·2
Dry weight (grm.)	0·904	0·569	0·150	0·058	0·013
Diam. (mm.) 4 days	17·7	16·0	16·2	16·4	16·0
6 ,,	25·0	24·0	23·9	23·7	23·7
8 ,,	31·3	31·6	31·1	30·6	31·0
12 ,,	40·3	46·4	48·1	47·9	47·6
14 ,,	(45)	54·8	57·1	56·4	55·7
Germ tube (2 trials)	1·1	1·5	1·5	1·4	1·9
	3·1	2·2	3·1	2·9	2·7

The graphs emphasize the fact that these three sets of data, which represent different aspects of growth processes, indicate a very different response to the environmental factor under consideration. High concentration, for instance, has little effect on spore germination, but is correlated in the subsequent stages of growth with a greater intake of food materials. At the same time the outward extension of the fungal colony, although little influenced at first, is in later stages retarded more markedly than with weaker media.

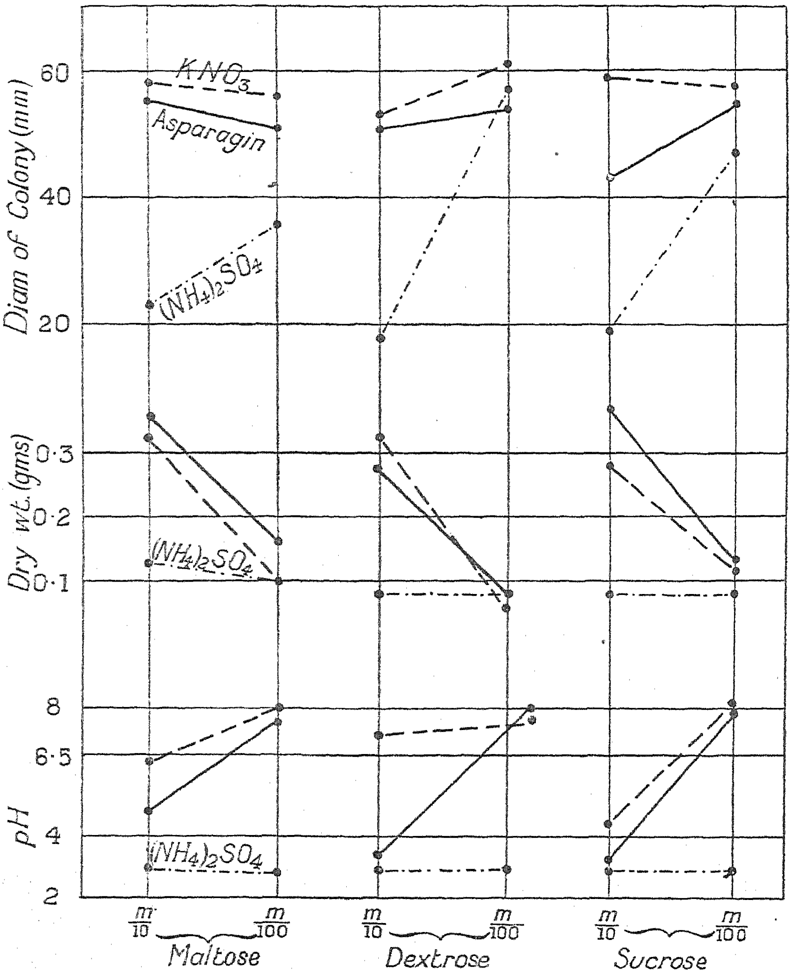


TEXT-FIG. 4. Graph showing the effect of concentration of medium upon fungal growth.

The Metabolism of Carbon and Nitrogen Compounds.

It has been shown repeatedly, during the course of the investigation, that *F. coeruleum* in liquid culture can readily make use of the commoner carbohydrates, viz. maltose, sucrose, dextrose, laevulose, starch, arabinose, and xylose, and can also use glycerol. The alkali salts of various organic acids were examined as to their availability as a source of carbon. It appeared that whilst sodium oxalate and formate are practically unavailable, growth develops freely on citrate, acetate, and tartrate. A further reference will be made to the utilization of these compounds.

Both asparagin and KNO_3 give good growth on various synthetic media. With both these compounds the medium tends to become alkaline, except when sugars are supplied in relatively high concentration. In the first table on p. 149 and in Text-fig. 5 are shown the results of a typical series of trials. It will be seen that as a general rule, with asparagin or KNO_3 at M/100, the medium tends to become acid with sugars at M/10, and alkaline with sugars at M/100, at M/20 the results being variable. The question of the relation between the reaction of the medium and the carbohydrate concentration was examined further by trials on liquid and agar media, using a series of concentrations of nitrogen compounds and sugar. In one such series asparagin and maltose were employed, and in another various proportions of KNO_3 and sucrose. The results are shown in the second and third tables on p. 149, and in Text-figs. 6 and 7.



TEXT-FIG. 5. Graph showing the relation between the nitrogen- and carbon-content of the medium and the growth of the fungus.

Growth of the Fungus on Media containing Asparagin, Potassium Nitrate, or Ammonium Sulphate (see Text-fig. 5).

Medium = S. + asparagin M/100	pH final.	Dry Weight (grm.).	Diam. (mm.). 11 days.	Diam. (mm.). 19 days.	Aerial mycelium.
+ maltose M/10	4·8	0·355	55	80 +	+
,, M/100	7·75	0·167	50·5	80 +	—
+ dextrose M/10	3·4	0·286	51·5	70 +	+
,, M/100	8·05	0·085	54·0	80 +	—
+ sucrose M/10	3·3	0·371	42·5	60	+
,, M/100	7·9	0·137	55	80 +	—
Medium = S. + KNO$_3$ M/50					
+ maltose M/10	6·35	0·330	58	90 —	—
,, M/100	8·1	0·105	55·5	80 —	—
+ dextrose M/10	7·3	0·358	52·5	80 —	+
,, M/100	7·6	0·062	61	80 —	—
+ sucrose M/10	4·4	0·286	59	80 —	+
,, M/100	8·2	0·130	57·5	80 —	—
Medium = S. + $(NH_4)_2SO_4$ M/100					
+ maltose M/10	3·0	0·133	23·5	23·5	—
,, M/100	2·9	0·105	36	57	—
+ dextrose M/10	2·9	0·080	18	19	—
,, M/100	2·9	0·080	57·5	80	—
+ sucrose M/10	2·9	0·075	18·5	19	—
,, M/100	2·9	0·091	47	70	(+)

Growth on Media containing various Concentrations of Asparagin and Maltose (see Text-fig. 6).

	Liquid.		*Agar.*			
Medium.	pH 16 days.	Colour.	Diam. (mm.). 6 days.	Diam. (mm.). 10 days.	Colour.	Aerial. mycelium.
S. + maltose M/100						
+ asparagin M/50	8·6	—	40·5	61·3	—	—
M/100	8·0	—	39·3	65·5	—	—
M/500	6·4	—	39·5	67·0	—	—
S. + asparagin M/100						
+ Maltose M/10	5·4	+ +	42·5	71·0	+ +	+ +
M/50	6·4	—	40·0	67·2	—	+
M/100	8·0	—	39·3	65·5	—	—
M/500	8·6	—	36·5	62·2	—	—

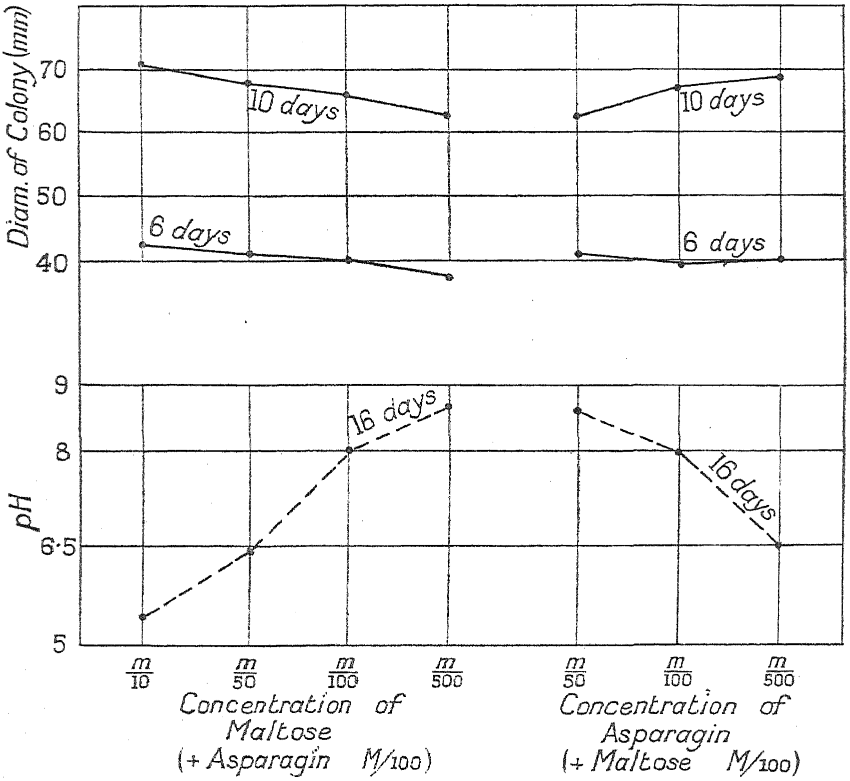
Growth on Media containing various Concentrations of Potassium Nitrate and Sucrose (see Text-fig. 7).

Medium.		pH final.	Dry Weight (grm.).	Colour.
S. + KNO$_3$ M/100 + sucrose	M/10	5·2	0·642	+ +
	M/50	7·7	0·197	+ +
	M/100	7·3	0·123	+
	M/500	6·9	0·019	—
	M/1000	6·7	(0)	—
S. + sucrose M/100 + KNO$_3$	M/100	7·9	0·105	+
	M/100	7·3	0·123	+
	M/5000	6·7	0·120	+ +
	M/100	6·0	0·101	+ +

As a general conclusion, it may be noted that:

1. The development of aerial mycelium (as opposed to surface film) is dependent upon a minimum carbohydrate concentration of not less than M/100.

2. Colour-production is largely independent of the amount of growth, but appears when the ratio of carbohydrate to nitrogen is high. The colour is usually purple or russet with asparagin, and indigo-blue with KNO_3. In agar cultures it appears first in the substrate, and later affects the aeria mycelium. The blue colour does not diffuse into the medium, although the liquid in flask cultures often becomes orange or pink.



TEXT-FIG. 6. Graph showing the relation between the asparagin- and maltose-content of the medium and the growth of the fungus.

3. The weight of mycelium varies with the sugar, and is little influenced by the nitrogen concentrations used. Sugar at M/100 sets a limit to growth with KNO_3 as low as M/1,000.

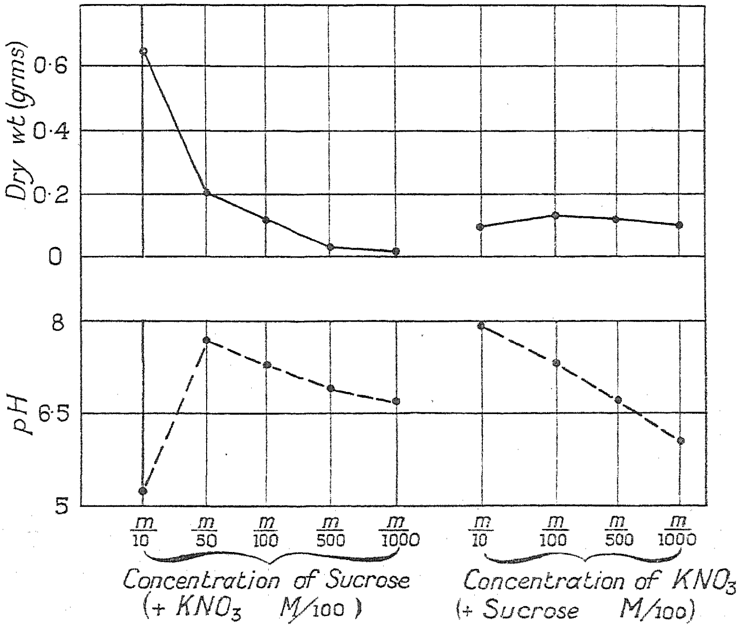
4. The rate of extension on a solid medium is not affected to the same extent as is the dry weight.

5. A tendency towards development of acidity in the medium is associated with a relatively high sugar concentration. When sugar is

relatively very low, the change of reaction may be slight, on account of the small amount of growth possible.

It appears that sugar concentration is the dominating factor throughout.

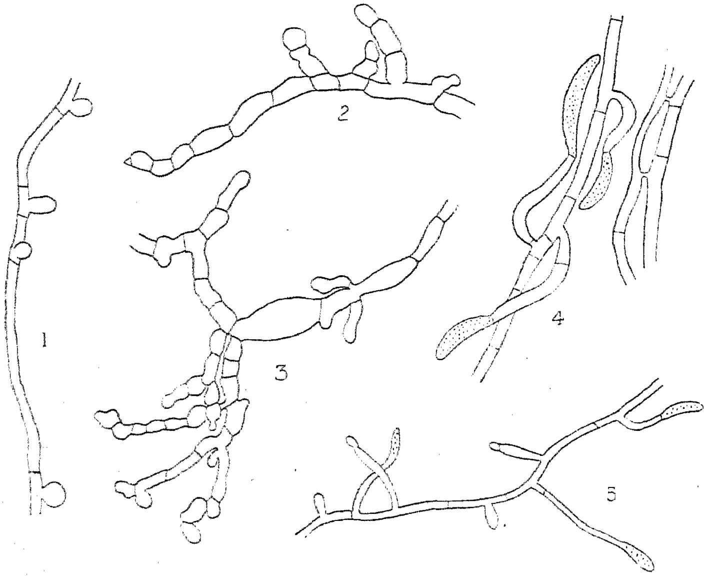
When nitrogen was supplied in the form of the ammonium salts of mineral acids, striking differences were observed. The utilization of such salts has been studied by Nikitinsky (16) and by Medisch (15). The former author, working with *Aspergillus* sp. and *Penicillium* sp., observed the liberation of free acid from ammonium salts of mineral acids. He also states that growth continues longer with NH_4NO_3 than with NH_4Cl or $(NH_4)_2SO_4$, and that a mixture of ammonium chloride and tartrate similarly remains longer favourable for fungal growth. The case of *Hypocrea* is interesting, since, according to Medisch, it is unable to assimilate sucrose in neutral media. With NH_4NO_3 and similar salts, the acid liberated is sufficient to invert the sucrose, and the resulting hexoses enable this fungus to grow. If, however, $CaCO_3$ be added to the medium, the acid is neutralized, and, since no inversion is possible, growth is prevented.



TEXT-FIG. 7. Graph showing the relation between the KNO_3- and sucrose-content of the medium and the growth of the fungus (39 days).

Similarly with NH_4Cl and $(NH_4)_2SO_4$, *F. coeruleum* can utilize the base and liberate the acid radicle. Striking results arise in consequence of the acidity developed. In extreme cases, the acid is sufficient to inhibit growth at an early stage, and the mycelium formed is of a remarkable and distinctive type. The colony on agar is small and deeply corrugated, with a firm, compact, and well-raised surface. The margin is crinkled, irregularly lobed or fringed, and growing deep in the agar.[1] The colour varies from orange to brilliant rose-red, and is much more pronounced in the submerged part of the colony. It passes out from the mycelium through the whole of the uninvaded part of the agar and becomes more intense throughout as the colony grows older. A typical colony is shown in Pl. VIII, Fig. 1.

The microscopic appearance of the mycelium is also peculiar. The hyphae show greatly increased septation, the swollen cells being frequently much enlarged and distorted. Abundant branching gives rise to densely-tufted clusters which are plainly apparent, even with the naked eye, in the fringed margin of the colony. No normal conidia or chlamydospores are formed, and the general appearance is that of a mass of rounded thin-walled spores budded off in chains. The development of this abnormal type of mycelium has been traced in drop-cultures of spores in media containing NH_4Cl and sucrose. Stages showing increasing septation and branching, as growth progresses, are represented in Text-fig. 8 (1, 2, and 3).



TEXT-FIG. 8. 1. Mycelium from young drop-culture on NH_4Cl+sucrose M/20, showing the tendency to branching and septation as the medium becomes acid. 2 and 3. Mycelium from similar, but older, cultures, showing increasing septation and branching as the medium becomes progressively more acid. 4 and 5. Mycelium from parallel cultures to which $CaCO_3$ had been added. Normal mycelium and conidia have developed.

It was found that the abnormal mycelium gave rise to the typical form, on transference to a more favourable medium. It was also plainly proved that the peculiar condition was due directly to the acid developed in the medium. Comparative trials were made with a medium containing NH_4Cl with the addition of $CaCO_3$ in suspension. In this case the normal type of colony is produced, with prolonged growth, typical aerial mycelium and conidia. The marked contrast in the external appearance of the colony (see Pl. VII, Figs. 3 and 4, and Pl. VIII, Fig. 2) is equalled by that in the mycelium grown in drop-culture (see Text-fig. 8 (4 and 5)). The following determinations were made in one such series of experiments; the media all being adjusted to pH 6·5 (approx.) at the beginning of the experiment:

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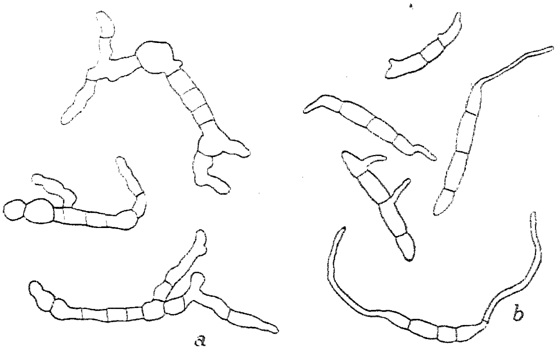
[1] Compare Brown's observation (4) of a similar occurrence in staled cultures of *Fusarium* sp.



TEXT-FIG. 9. (*a*) Conidia germinating in medium adjusted to pH 3·0–3·5. (*b*) Normal germination of conidia in medium adjusted to pH 5·5. (× 400.)

Growth on Media containing Ammonium Sulphate with or without the addition of Calcium Carbonate.

Medium = S. + sucrose M/10	pH final.	Dry weight (grm.).	Diam. (mm.) 7 days.	Diam. (mm.) 16 days.
+ $(NH_4)_2So_4$ M/100 alone	3·0	0·085	13·7	15·5
+ „ „ + $CaCO_3$	7·1	0·195	32·7	48·2
+ $(NH_4Cl$ M/50 alone	3·0	0·072	(12)	14
+ „ „ + $CaCO_3$	7·2	0·277	30 + 35	50 + . . .

It has already been shown that the degree of acidity here developed (pH 3·0) is near the maximum at which spore-germination is possible. Conidia incubated in a medium of slightly less acidity showed a similar response to the reaction and germinated slowly, producing a swollen, septate, and much-branched germ tube. In Text-fig. 9 are represented the types of germination obtained in media adjusted to pH 3·3 and 5·5 respectively.

With media containing NH_4Cl or $(NH_4)_2SO_4$, the amount of acidity produced and the degree of the consequent morphological change are much influenced by the sugar concentration. With sugar M/10, the limiting degree of acid is soon attained; in consequence, growth is stopped at an early stage, and the fungus shows the extreme type of acid response described above. With sugar M/100, however, the acid liberation is limited by the reduced carbohydrate metabolism; consequently the colony grows for a longer time and is less extreme in type. On agar it shows a flat, even surface with broadly-lobed margin and increased septation. In flask cultures the typical acid appearance is developed. As in other cultures containing sugar M/100, no colour appears. Typical results of one series of comparative trials are given on p. 149.

With NH_4NO_3 good growth is obtained and the medium becomes only slightly acid, indicating that the compound is largely used as a whole. $(NH_4)_2CO_3$ behaves in the same way, and continued growth is possible also on ammonium oxalate (M/100).

It has been shown that sodium citrate can be utilized as a source of carbon supply, with liberation of alkali. When it was added to a medium containing $(NH_4)_2SO_4$ and sucrose M/20, no free acid was developed and abundant growth was therefore possible.

Growth on a Medium containing Ammonium Chloride and Sodium Citrate.

Medium = S. + sucrose M/50	pH.		Dry weight (grm.).
	Initial.	Final.	
+ NHCl alone	6·4	3·0	0·090
+ Na citrate	5·6	9·3	0·271

Sodium tartrate and acetate behaved in the same ways in combination with both ammonium salts and KNO_3.

Effect of Varying Concentrations of Phosphate.

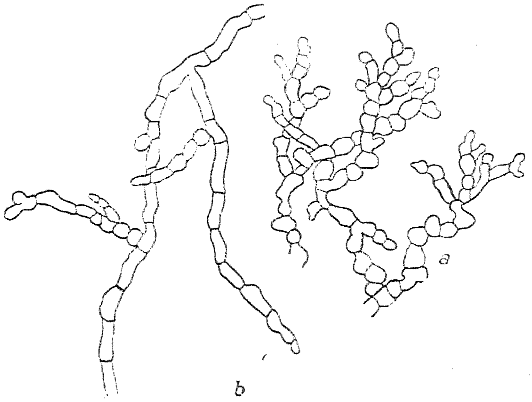
In general KH_2PO_4 at M/500, M/100, or M/20 seems to have little influence on the habit and amount of growth, these being determined primarily by the nitrogen and carbohydrate proportions. Trials have been carried out, using KH_2PO_4 at various concentrations, combined with sucrose (M/20 or M/200) and different nitrogen compounds (see Pl. VII, Figs. 4 and 5, and Pl. VIII, Figs. 2 and 3). Only with NH_4Cl was any noticeable difference to be ascribed to the varying phosphate. Here the development of high acidity (with sucrose M/20) was delayed by increasing the phosphate, so that the colony reached a larger size before growth was finally stopped. This may perhaps be due to the buffer action of the phosphate. The variation shown in external appearance can be correlated with corresponding differences in the hyphae as seen under the microscope (see Text-fig. 10).

F. coeruleum as a Pathogenic Organism.

1. *Its action on the cell-wall.*

Previous workers have indicated the means by which this fungus brings about the rot of the potato after its entry through a wound. Pethybridge has stated that the parenchyma cells are first separated from one another, the hyphae penetrating between them and entering them only at a later stage of decay. Hawkins (10) has found that *F. oxysporum* and *F. radicicola* (both of which can produce rotting of the tuber) reduce the pentosan-content of the tuber and secrete an enzyme which can hydrolyse xylan. In this relation, however, he makes no statement regarding *F. coeruleum.*

By employing methods similar to those used by Brown (2) with *Botrytis*, it has been possible repeatedly to demonstrate the actual secretion, by *F. coeruleum*, of a cytase which is capable of dissolving the middle lamella of the cell-walls of tuber-tissue. The fungus was grown in liquid media (Richards' solution or extracts of potato or turnip), and it has been found that tuber-tissue was disintegrated both by the culture fluid and by the aqueous extract of the dried mycelium. The potato discs used for the test lost coherence after a few hours' treatment, the time varying from three hours upwards at laboratory temperature.



TEXT-FIG. 10. (*a*) The extreme 'acid' type of mycelium developed on agar medium containing NH_4Cl + sucrose M/20 + KH_2PO_4 M/500. (*b*) The less markedly 'acid' type of mycelium developed in a similar medium containing KH_2PO_4 M/20. (Compare Plate VII, Fig. 4.)

With young potato shoots also, the inner tissues are disintegrated by the action of an extract injected into them. The lignified tissues are resistant and stand out conspicuously amongst the disorganized débris. It has been found, on the other hand, that the storage parenchyma of carrot, turnip, and onion are markedly resistant both to the lethal and macerating action of the extract. The skin of the tuber is also unaffected.

2. *Its action on cell contents.*

Hawkins (l. c.) found that *F. coeruleum* secreted enzymes capable of hydrolysing maltose and sucrose. The production of invertase has been confirmed during the present investigation.

It has been pointed out by Pethybridge that the starch grains of affected tubers are not attacked. His statements are supported by the chemical analyses of Hawkins, who found that the total starch-content of the tuber was not reduced by the invasion of the fungus. During the present study it has been repeatedly observed, both in drop-cultures and in enzyme tests, that raw potato-starch is unaffected either by the fungal mycelium or by its extract. Gelatinized starch is, however, readily hydrolysed, and so also is the soluble product obtained by grinding raw potato-starch with sand or kieselguhr.

It may here be pointed out that Day (quoted in Reichert's Memoir, p. 191) (19) recognizes two constituents in the potato-starch grain: (1) an outer resistant covering, red amylose; (2) a more digestible inner substance, blue amylose. Neither constituent becomes more digestible on boiling, and he therefore concludes that the character of the outer part is responsible for the marked resistance of raw potato-starch to the action of many diastatic preparations. This is in agreement with Maquenne's statement (Reichert's Memoir, p. 178) that thorough grinding is as effective as heat gelatinization in rendering raw starch digestible.

The Parasitism of F. coeruleum.

It is well known that the species of *Fusarium* which attack stored potatoes do so in general more vigorously during the later stages of the rest period of the tuber. Pethybridge has proved this point by controlled inoculations at different times of the year. He has further shown that there is a marked difference in the susceptibility of different commercial varieties.

Many inoculations were carried out during 1920–3, with several varieties. The tubers were in all cases first washed with mercuric chloride or formalin, rinsed with sterile water, and then inoculated, through one or more wounds, with a fragment of mycelium or a drop of a spore suspension. The tubers were stored in covered vessels and examined finally after an interval of several weeks. As with some other Fusarial rots, the type of decay varies somewhat with the conditions under which the potato is stored after inoculation. Under the usual field conditions, and if kept in a dry jar, the tuber dries considerably, and the skin over the infected areas shows concentric wrinkles (see Pl. VIII, Fig. 5); but if the tubers are stored in a saturated atmosphere a wet rot is produced. In either case the inner tissues break down to a pulp of disintegrated cells, the characteristic blue colour appears locally, and cavities are formed lined with white mycelium (see Pl. VIII, Fig. 6). The skin is unbroken until the rot is well advanced, when white or cream-coloured spore-bearing pustules break out (see Pl. VIII, Fig. 4).

The results (given below) of comparative inoculation trials indicate that the varieties Ninety-fold and May Queen are markedly more susceptible than Epicure. In Pethybridge's trials of nine different varieties, Ninety-fold appeared to be the most susceptible and Epicure the most resistant. His figures for successful infections are: Ninety-fold 100 per cent., May Queen 80 per cent., Epicure 2 per cent.

Results of Inoculation Experiments with different Varieties of Potatoes.

Variety.	*Date.*	*No. of inoculations.*	*No. of infections.*	*% of infections.*	*Culture used.*
May Queen	April 28, 1921	16	16	100	C
King George	,,	18	1	5-6	,,
Snowdrop	,,	22	6	27	,,
Ninety-fold	,,	24	24	100	,,
Epicure	Mar. 3, 1922	11	0	0	,,
Edzell Blue	,,	10	1	10	,,
Ninety-fold	,,	10	8	80	,,

			No. of infections.			
			Definite positive.	*Doubtful.*	*Negative.*	
Epicure	Dec. 1922	20	7	5	8	4
Ninety-fold	,,	20	18	2	0	,,
Epicure	Feb. 1923	20	0	10	10	,,
Ninety-fold	,,	19	19	0	0	,,

The existence of seasonal variation in susceptibility has also been confirmed by the results of a large number of inoculations performed at intervals through the storage period. The results are given below.

Results of Inoculations of 'Ninety-fold' Tubers at different Seasons.

Variety.	*Date.*	*No. of inoculations.*	*No. of infections.*	*% of infections.*	*Culture used.*
Ninety-fold	Dec. 20, 1920	55	1	18	C
,,	Apr. 12, 1921	16	6	37	,,
,,	,, 28, ,,	24	24	100	,,

It should be mentioned that the Amsterdam strain (C) used during the earlier part of the investigation was not very vigorously pathogenic. The new strain (4) was found, in repeated comparative trials, to be a much more vigorous parasite. This difference is perhaps to be correlated with the fact that Strain C had been kept for ten years on artificial media, whilst Strain 4 was freshly isolated from a rotted tuber. In Pethybridge's investigation also, the Amsterdam strain used by him appears to have been less pathogenic than his freshly isolated strain.

The existence of both a varietal and seasonal difference in susceptibility is more easy to demonstrate than to explain. The problem has been attacked from various standpoints in the attempt to correlate a difference in susceptibility with some constant difference in the character of the tubers concerned.

It is possible that resistant tubers contain some substance which affects the growth of the fungus. The experiments of Pethybridge indicate that difference in sugar-content does not affect the liability to attack. In the course of the present work the H-ion concentration of the expressed juice of many tubers has been determined, but the maximum difference observed (from pH 5·8–6·2) is negligible in comparison with the wide range of reaction readily tolerated by the fungus. An examination has been made of the germination of spores in the expressed juice of tubers of different sorts. Technical difficulties are involved, since the drops of juice darken and precipitate heavily during incubation, so that accurate measurement of the germ tube is impossible. This difficulty can be avoided either by heating the juice to 80° C. (at which temperature the proteins are precipitated and the colour change does not occur), or by filtering the raw juice through a porcelain thimble. Both methods were tried, and in neither case was any constant difference found in the lengths of germ-tube developed in the sap of the tubers under comparison. It is, of course, uncertain what alteration is produced by either treatment, even in the non-protein constituents of the juice.

Again, it is possible that tubers may show differences in the behaviour of the middle lamella of their cell-walls towards the solvent action of the fungal secretions. Differences in the character of the middle lamella have been demonstrated by Valleau (22) for certain varieties of plum, and have been correlated with their degrees of resistance to the invasion of *Sclerotinia*. Many attempts were made to show whether a cytase preparation of *F. coeruleum* exhibited any constant difference in its action towards susceptible and resistant tubers. The potato-disc method already mentioned was used, and comparison was made of the length of treatment required to produce disintegration. The tubers selected for examination were of the varieties Ninety-fold and Epicure, and were in all cases tested simultaneously and with the same enzyme extract. No constant difference could be detected either between new and old tubers of the same variety or between tubers of the same age of the two varieties examined. If such differences exist at all, they cannot be demonstrated by this experimental method. It is nevertheless of interest to recall the observation (p. 155) that sections of carrot, turnip, and onion are very resistant to the action of the extract. It has been shown by Pethybridge, and again by the author, that these plants cannot be infected by *F. coeruleum* under the usual conditions of experiment.

Yet again, it is possible that, since this fungus is a wound-parasite, the susceptibility of tubers may be dependent on the nature of the wound-cork or on the rapidity of its formation. Olufsen (17) has pointed out that immature tubers react more quickly to wound-irritation, whilst Shapovalov and Edson (20) consider that the delay of cork-formation in shrunken tubers is due to the reduced water-content. In this connexion it may be noted that the fungus could produce a rot in the susceptible variety Ninety-fold if inoculated on to a wound seven days' old. The rot was slower in developing and less certain than with inoculation on to a fresh cut wound. With the variety Epicure no infection resulted if the wound was more than three days old. Both from this and from other inoculation trials there is an indication that the fungus is slower in *starting* its invasion of the resistant variety.

SUMMARY OF RESULTS.

1. The rate of growth of *F. coeruleum* at different temperatures has been examined by determination of (*a*) dry weight of mycelium in liquid culture, (*b*) diameter of colony on solid media, (*c*) the length of germ tube of conidia in germination drops. The maximum lies at or near 30° C., whilst the minimum is slightly below 5° C. The optimum is between 15 and 25° C., and probably nearer 20° C. than 15° C.

2. Conidia are killed by heating for 10 to 15 minutes in water at 45–46° C., or for a longer time at a lower temperature. Where the treatment is insufficient to kill the spores, their germination is, however, delayed to an extent which varies both with the duration and the intensity of the heat applied.

3. The maximum acidity tolerated by the fungus is near pH 3·0. Growth is still possible at pH 10·5. There is a marked morphological change as a result of growth between pH 3·0 and 3·5.

4. The carbon compounds utilized by the fungus include the commoner carbohydrates, glycerol, sodium salts of tartaric, citric, and acetic acids. Oxalates and formates are not utilized.

5. Potassium nitrate, asparagin, and the ammonium salts of certain organic and inorganic acids can serve as a source of nitrogen to the fungus.

6. The concentrations (absolute and relative) of the substances utilized by the fungus as sources of nitrogen and carbon profoundly affect the reaction developed in the medium, and hence influence the amount and type of the fungal growth. The concentration of phosphate has relatively little influence.

7. The fungus secretes diastase, invertase, and cytase.

8. The existence of both varietal and seasonal differences in the susceptibility of tubers has been confirmed. The difference appears to be due neither to varying sugar-content nor to differences in acidity of the expressed juice. Neither can it be attributed to any difference in the cell-walls of susceptible and resistant tubers when acted upon by a fungal extract.

I have great pleasure in here recording my indebtedness to Prof. V. H. Blackman for his continued interest in this work, the greater part of which

has been carried out under his guidance at the Imperial College of Science, partly with the aid of a grant from the Ministry of Agriculture. The investigation has been completed at the University College, Southampton, where Prof. Mangham has kindly placed at my disposal every facility of his department.

UNIVERSITY COLLEGE,
SOUTHAMPTON.

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EXPLANATION OF PLATES VII AND VIII.

Illustrating Dr. Moore's paper on the Physiology of *Fusarium coeruleum.*

PLATE VII.

Fig. 1. Two colonies developed on agar at 30° C. (above) and 25° C. (below) respectively.

Fig. 2. Colonies developed on agar media containing a nutrient (Coons' solution) at a range of concentrations.

Fig. 3. Two colonies developed on media containing ammonium chloride and sucrose (M/10). To the medium in the upper dish calcium carbonate also has been added.

Fig. 4. Colonies showing the development of the 'acid' type, modified by increased phosphate.

Fig. 5. Colonies showing only a very slight tendency towards the 'acid' type (the consequence of low sugar concentration), which is further modified by increase of phosphate.

PLATE VIII.

Fig. 1. A typical colony (slightly enlarged) developed on a solid medium containing ammonium sulphate and a high concentration of sugar.

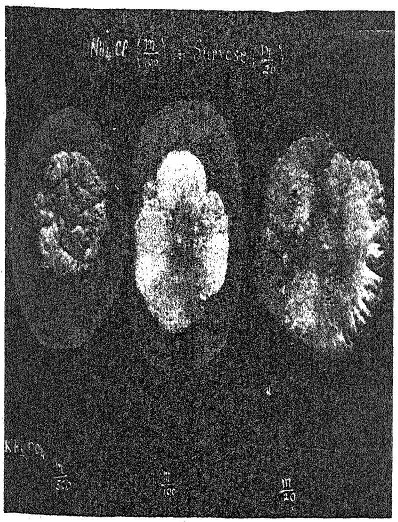
Fig. 2. Colonies developed on media similar to those of the cultures shown in Plate VII, Fig. 4, with the exception that calcium carbonate has been added.

Fig. 3. Colonies developed on media similar to those of the cultures shown in Plate VII, Fig. 4, with the exception that NH_4Cl has been replaced by KNO_3.

Fig. 4. External appearance of artificially-inoculated potatoes, kept under moist conditions, and showing an advanced stage of rotting.

Fig. 5. External appearance of a tuber artificially inoculated in two places, showing the typical shrinkage of the infected areas, with concentric wrinklings of the skin.

Fig. 6. Internal appearance of inoculated potatoes, showing the discoloration of the tissues and the formation of cavities lined with mycelium.


$NH_4Cl \left(\frac{m}{100}\right) + \text{Sucrose} \left(\frac{m}{20}\right)$
KH_2PO_4
$\frac{m}{500}$
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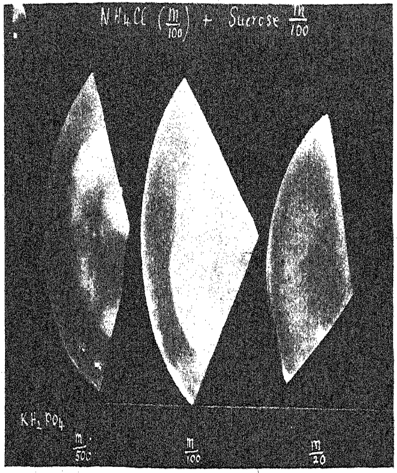
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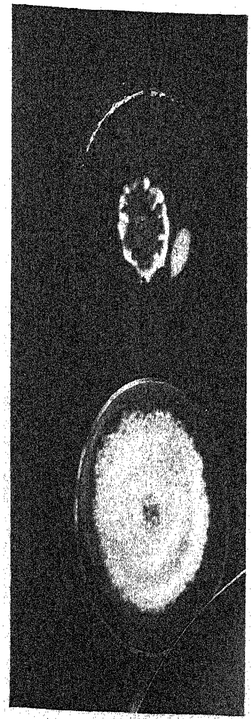

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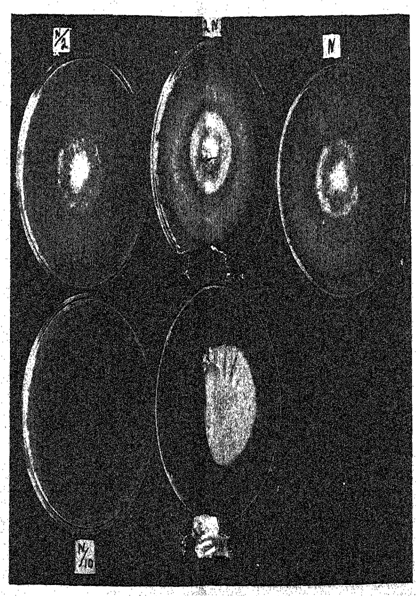

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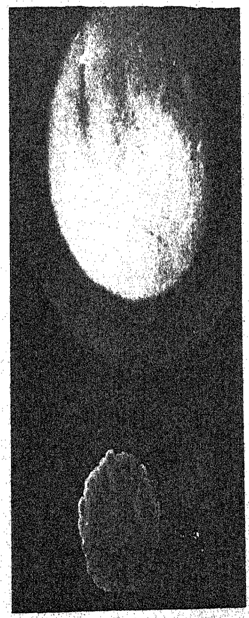

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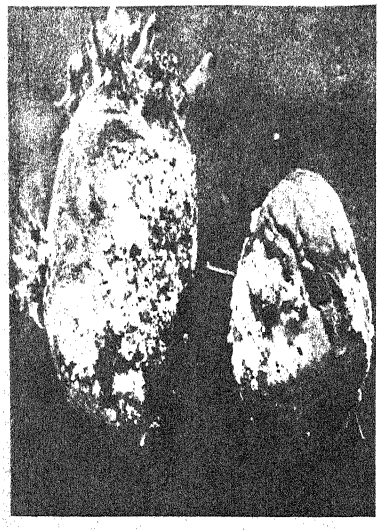

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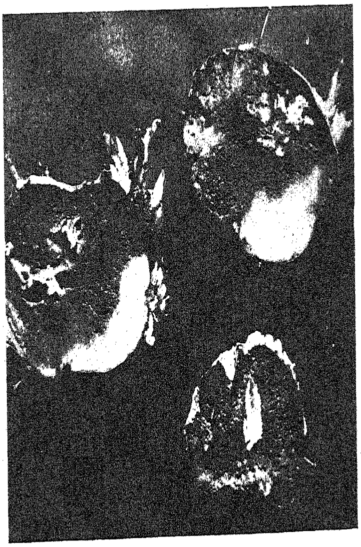

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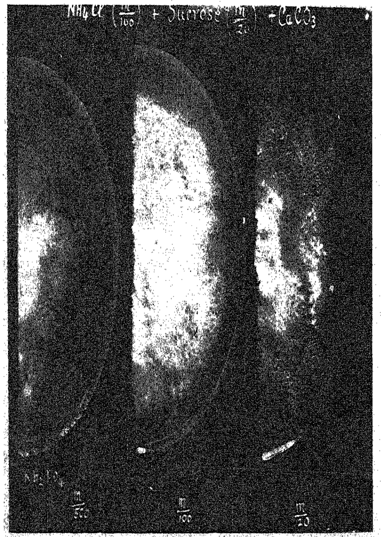
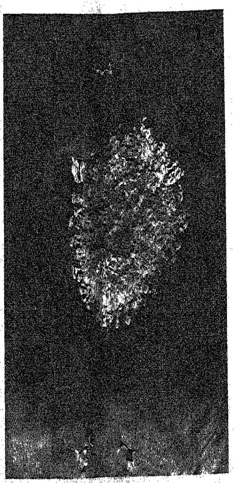


$NH_4Cl\ (\frac{m}{100})$ + Sucrose $(\frac{m}{20})$ + $CaCO_3$
KH_2PO_4
$\frac{m}{500}$
$\frac{m}{100}$
$\frac{m}{20}$

2.



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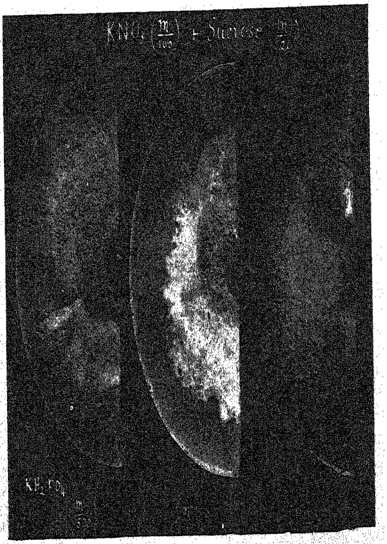

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Further Experiments on the Conduction of Tropic Excitation.

BY

R. SNOW.

With six Figures in the Text.

IT was recently shown that when the tip of a decapitated root is stuck on again with gelatin the root becomes again capable of curving down in response to gravity (Snow, 1923). But it could not be determined with certainty whether this curvature is due to excitation transmitted back across the gelatin from the tip, since the stimulus of gravity cannot be applied to the tip alone. It was therefore decided to make similar experiments with traumatic stimulus, with which there is no such difficulty. Further, since it had been found that excitations leading to tropic responses can be conducted back along either the (prospectively) convex or the concave side of the root alone, it seemed desirable to compare quantitatively the effects of these two excitations. Certain other experiments also, for which the root is not suitable, have been carried out on the cotyledon of *Avena*. The theory of 'straight-line conduction', previously supported, will be adopted as a working hypothesis, and further discussion of it postponed to the end of the paper.

1. EXPERIMENTS ON ROOTS OF *VICIA FABA*.

Methods. For experiments on roots, *Vicia Faba*, the broad bean, was again made use of throughout. Different races of broad bean differ greatly in sensitivity. The age of the seed is also apparently of importance, but this point is still obscure. In the previous experiments on geotropism Taylor's Broad Windsor was chiefly employed, and found sensitive and straight-growing. But to traumatic stimulus this race responds less vigorously than Aquadulce Long-pod and Johnson's Long-pod. In the following decapitation experiments Aquadulce was employed. But for the experiments with mica plates Johnson's Long-pod was found better, since this race and Taylor's Broad Windsor are much less apt to deviate as

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the result of a lateral incision than is Aquadulce. The root of *Vicia Faba* has been considered to respond too feebly for traumatotropic experiments (Günther, 1913). But with the above two races, curvatures of 90° or more could be obtained. Several other races were found less suitable. After replacement of the tip with gelatin, the root was dipped into melted cocoa butter, up to just above the joint. This prevents the tip from being washed off by water secreted from the stump.

For traumatic stimulation, a glass rod, heated so as just to give a sodium flame, was applied lightly for an instant to the side of the tip at the level of the vegetative apex. A dark semicircle is at once seen, under the lens, to spread out from the point of stimulation. This is due to the local injection of the intercellular spaces of the periblem with water presumably excreted by the stimulated cells. Response seems to be greatest when the stimulus is so adjusted that this semicircle reaches just about to the plerome. It must not spread across to the far side of the tip. Other methods of stimulation were less effective. When inspected later, the tissue within this area is found to be dead, and sharply marked off from the surrounding tissues.

Experiment 1.

In all, 25 roots of Aquadulce beans were decapitated at 2·5 mm. from the vegetative apex, and the tips, while still adhering to the knife, were stimulated on one side with a hot glass rod. They were then replaced with gelatin, and the beans pinned with the roots vertical.

Of these, 9 remained straight; 16 curved away from the stimulus, in curves from 5° to 30°, the average being $15\frac{1}{2}°$; none curved towards the stimulus. Although the curvatures were not very vigorous, this result shows that, after traumatic stimulation of the tip, tropic excitation can be transmitted back from it through a layer of gelatin. The probability that geotropic excitation also passes through gelatin is thereby increased.

In similar experiments, however, carried out on a very few roots of Johnson's Long-pod, no curvatures were produced. The reason for this difference is not clear.

Experiment 2. Comparison of the efficiencies of the two sides of the root in conducting excitation.

The following experiments were carried out both with traumatic stimulus and stimulus of gravity, and with two races of bean, Taylor's Broad Windsor (T.B.W.) and Johnson's Long-pod (J.L.P.). Since the extents of curvature differ widely in different individuals, probable errors must be calculated before the two sides can be compared.

Before stimulation, conduction along one side was prevented by insertion from that side of a mica slip to a depth of half-way through the root, at 2·5 mm. from the vegetative apex with T.B.W., and 2 mm. with J.L.P. To secure uniformity of conditions, equal numbers of roots with slips on each of the two sides were, so far as possible, made use of on each occasion. Since the insertion of the slip may itself cause some curvature, the extent of this must first be determined by controls and then allowed for.

The following measurements refer to curvatures above the cut only. The temperature was about 19° C.

TABLE OF RESULTS.

(*A*) *Controls.*	*Number of roots.*	*Mean curvature* (*d*).	σ.	σ *of the mean* (σ_d).
J.L.P. slip at 2 mm.	10	7° towards slip	9·16°	2·9°
T.B.W. slip at 2·5 mm.	11	2·27° towards slip	8·1°	2·44°

	(1) *Number of roots.*	(2) *Mean curve.*	(3) *Mean curve corrected for 'd'.*	(4) σ.	(5) *Difference of corrected means.*	(6) *Chance of such difference arising from error of random sampling.*
(*B*) *J.L.P. Traumatic stimulus.*						
Slip on same side as stimulus	19	−30·8	−37·8	25·2	24·1 ± 5·8	1 in 185
Slip on far side	22	−20·7	−13·7	12·6		
(*C*) *T.B.W. Traumatic.*						
Slip on same side	13	−24·6	−26·9	21·0	11·9 ± 5·5	1 in 6·4
Slip on far side	13	−17·3	−15·0	11·2		
(*D*) *J.L.P. Stimulus of gravity.*	(Roots laid horizontally.)					
Slip above	18	−39·7	−46·7	20·9	26·0 ± 6·0	1 in 250
Slip below	18	−27·7	−20·7	19·7		
(*E*) *T.B.W. Stimulus of gravity.*	(Roots laid horizontally.)					
Slip above	21	−26·3	−28·6	17·3	14·6 ± 5·7	1 in 12·2
Slip below	19	−16·26	−14·0	25·0		

(Curvatures away from stimulus or downwards are reported as negative.)

From the above tables it is clear that in all cases conduction along the side that will become concave leads to a greater mean curvature than conduction along the side that will become convex, and further that this difference is enough to be significant.

The method of calculating the chance that the difference may be due to error of sampling may be illustrated in the case of (*B*) above.

For the standard error of the difference of the means, we have

$$E_{1-2}^2 = \frac{\sigma^2}{n_1} + \frac{\sigma^2}{n_2} = 40\cdot74 \qquad \therefore E_{1-2} = 6\cdot38.$$

But allowance must also be made for the standard error of '*d*'.

From the controls $\sigma_d = 2{\cdot}9$.
But the correction for '*d*' is applied twice, being added to the results with mica slip on the same side as the stimulus, and subtracted from those with the slip on the far side.

∴ Total standard error for difference of corrected means is

$$\sqrt{E_{1-2}^2 + (2\sigma_d)^2} = 8{\cdot}6°,$$

∴ total probable error $= 5{\cdot}8°$.

The chance that the observed difference of the means (x) might arise as an error of random sampling can now be calculated from tables giving the areas under the normal probability curve on either side of ordinates at abscissae $\frac{x}{\sigma}$.

In the case of traumatic stimulus, it is of course known on which side of the tip the stimulus first acts. With stimulus of gravity this is not known, although this stimulus too must act differently on the two sides of the tip, if curvatures are to follow. But since, in both cases, the distribution of conducted excitation is similar (the concave side being the more effective) it is suggested that the stimulations also may be similarly distributed, gravity acting primarily on the upper side of the tip. This appears more clearly in the *Avena* cotyledon, in which, as shown by Miss Purdy (1921), the far side conducts about six times more effectively in phototropism, while the lower side is the more effective in geotropism. Here, too, it may be suggested that gravity stimulates mainly the upper side of the tip.

Since it appears that there arise in the root two excitations, of opposite sign, conducted along the two sides, it would be desirable to know which of these can travel by diffusion across a protoplasmic gap (as in Expt. 1) or whether both can do so. An attempt was made to answer this question by applying stimulus after making cuts from opposite sides, and blocking one with mica while the other was left unblocked. Unfortunately the double cuts lead by themselves to so many irregular curvatures that the attempt had to be abandoned until more favourable material could be found.

That, in the traumatotropism of the root, the sides farther from the applied stimulus should conduct back excitation more effectively than the near side is certainly at first surprising. But a closely comparable state of things holds for phototropic conduction in *Avena*. It appears as if the primary traumatic stimulation on the near side of the root-tip brought about a secondary excitation of opposite sign on the far side of the tip, from which the far-side conduction would originate. It may be noted that Nemec (1901, pp. 46–7) was led by the visible changes in the root-tip after traumatic stimulation to distinguish a primary excitation, capable of spreading transversely, though slowly, and a secondary excitation only conducted longitudinally, and originating from cells affected by the primary excitation. But the connexion between these appearances and the curvatures of response remains doubtful.

2. PHOTOTROPIC EXPERIMENTS WITH *AVENA* COTYLEDON.

Methods. In the earlier experiments a black Tartarian oat (*Avena orientalis*) was used, but in the later the 'Abundance' variety of *Avena sativa*. No difference was noticed in the behaviour of the two races. The plants were grown in jars of sawdust in an electrically-heated box lined with black paper, in a dark room. The temperature within the box was about 24° C., and the air within it was kept moist.

If strong curvatures are to be obtained without the use of the klinostat to eliminate geotropism, the stimulating light must be applied continuously. But the high intensities of light commonly used seem unnecessary. An intensity of from 1 to 2 metre-candles was found enough to keep the cotyledons very strongly curved in opposition to geotropism. Accordingly arrangement was made to stimulate the plants within the heated box with a small electric bulb, at about 30 cm., adjusted to give about $\frac{1}{5}$ candle-power. The use of such low intensities makes it far easier to prevent errors due to accidental access of light to the lower zones of the cotyledon when these have to be kept in darkness.

The operations were carried out by red light passed through a spectroscopically tested screen.

Experiment 3.

It was desired to determine in which direction the lower zones curve if the tips alone were stimulated, after they had been cut off and replaced in such a way that the far side of the tip made contact with the near side of the lower zones.

The cotyledons were decapitated by the ingenious method of Stark (1921). A nick was made with a scalpel on one side at 4 to 5 mm. below the tip, and then the brittle cotyledon was broken at the nick by bending gently, much as a glass tube is broken by the chemist. The tip can then be pulled off, while the flexible included leaves are left projecting. A pull on these latter will then sever them at their base; if this is not done, by their further growth they knock the replaced tips off again. In the present case, the included leaves were not completely removed, but, after severance at the base, were left projecting. They were then painted on both sides with a dilute solution of gelatin, as were also the ledges at the top of the stump. The tip, previously removed, was then carefully replaced on the ledge at one side, and rested up against the projecting leaves, to which it adhered (Fig. 1). It is this adherence to the included leaves that makes it possible to balance the tip on the ledge, which is only about 0·3 mm. wide.

Of the plants thus operated on some were kept in darkness as controls, and others stimulated on the tip only with light. The direction of the light is shown by the arrow in Fig. 2. The lower zones were kept darkened by shields of tin-foil.

At first, difficulty was found in regulating the moisture of the air. If the stumps excrete drops of water, the tips are washed off; if the moisture falls much below this point, they begin to wilt. The right moisture was finally secured by suitable arrangements of bell-jars, lined with black paper except on one side. Results are reported only for plants of which the tips neither fell off early nor wilted.

Results after 5–7 hours. (Curvatures away from the side on which the tip rests are marked as −. In *B* this is also the direction away from the light.)

A. *Controls.*	*B.* *Stimulated plants.*
0	0
0	−18°
−5	0
−5	0
−5	−20°
+5	0
0	0
0	−18°
0	0
0	−5°
0	0
+15	0
0	−10°
0	−20°
Mean = +0·36°	Mean = −6·5°

Difference of mean curvatures of *A* and *B* = 6·86 ± 1·72°. Chance that difference may be due to error of random sampling = 1 in 139.

In the above table the negative results under *B* are certainly numerous. But it should be remembered that it is not easy to ensure a good joint between the tip and the ledge below it, so that success in every case was not to be expected, and the mean curvature does not adequately represent the results obtained. I do not think that any one who had seen the curvatures in *B* would doubt that they were significant. The appearance was particularly striking, since the tips were curved as in a normal response towards the light, in abrupt contrast to the lower zones, which were obviously curved away (Fig. 2). It was, however, a possibility that similar curvatures of the lower zones, away from the side on which the tip was placed, might occur even without stimulation by light; but the controls show that this is not the case.

It is thus clear that the phototropic excitation transmitted back from the far side of the tip does not necessarily cause curvature towards the light, but simply causes relative expansion in that side of the stump down which it passes. If it passes down the far side of the stump, as in the intact plant, the stump curves towards the light; if down the near side, as in the above experiment, it curves away.

Experiment 4.

In five plants cuts were made half-way through, below the tip from opposite sides, and the tip alone stimulated with light, the lower zones being shielded. The cut nearest the light was blocked with a mica slip, while that on the far side was not blocked. The plants were kept in damp air. Plants in which the included leaves had not reached up to the tip were most suitable, as in them the mica slips do not get pushed out of place.

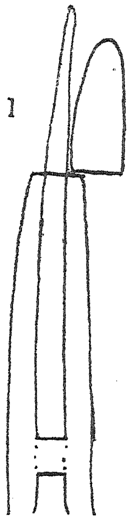


FIG. 1.

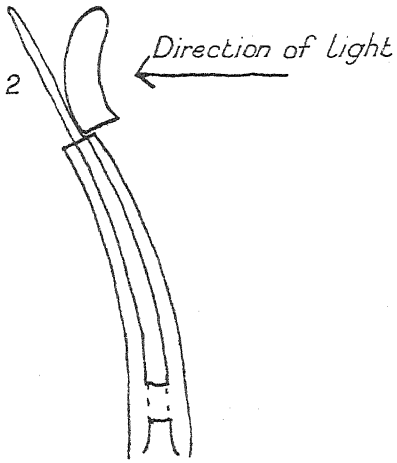


FIG. 2.

After 5–7 hours, four plants were all curved towards the light, while one remained straight.

This result, if conduction takes place along straight lines, only shows that the excitation conducted down the far side must be able to pass across a moist gap. It was already probable that it is this excitation that can pass through gelatin, as in the original decapitation experiment of Boysen-Jensen (1910), since it is six times as effective as the near-side excitation (Purdy, 1921); this conclusion was also reached by Boysen-Jensen. It would be desirable to know whether the excitation conducted along the near side can also pass a gap or not; but in *Avena* this excitation is relatively so feeble that there seemed little hope of deciding the question. It may be mentioned that the original decapitation experiment was again repeated with positive result.

DISCUSSION.

With regard to the conduction of tropic excitation, there are current two conflicting views. The simpler of these is that the stimulus acts differently on the two sides of the perceptive region. Thus different excitations or degrees of excitation are set up and conducted back along straight lines to the corresponding two sides of the responding region, which they affect differently. Thus curvature follows in the plane of the stimulus.

On the other hand, Fitting (1907) considered that he had shown, by elaborate experiments on *Avena*, that this simple view was inadequate, since the excitation could travel by sinuous paths, and for other reasons. He therefore concluded that conduction involves the spread of some 'polarized' condition of the protoplasm, the polarization being oriented with reference to the stimulus. The details of this difficult conception are less important than the question whether his experiments are conclusive. Now whether or not such 'polarized' conduction exists, it seems certain that conduction of the kind contemplated by the 'straight-line' theory does take place. For, first, conduction can in many cases pass through a gelatin layer, through which the transmission of any 'induced polarity' seems hardly possible. And, secondly, it is clear from Expt. 3 above that in the case of the far-side phototropic excitation in *Avena* the direction of response depends not on any polarity in the conducted excitation, but only on its distribution in the organ. The far-side excitation transmitted from the tip simply leads to relative expansion in that side of the lower region (whichever it may be) down which it passes.

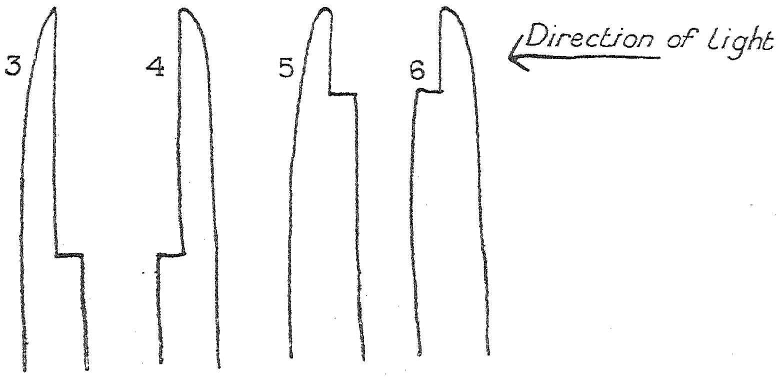
But though such conduction seems established, it is not excluded that a 'polarized' conduction of some quite different kind might also take place, and show its effects under certain conditions. It remains, therefore, to see whether Fitting's experiments make it necessary to believe that this is the case. In reading of these experiments one cannot but be impressed by the thoroughness of the investigation and the elaborate and scrupulous precautions observed. Moreover, he worked at a higher temperature (29° to 31° C.) than other investigators. But it should be remembered that at that time the possibility of conduction in plants across a watery gap had not been discovered; had it been, the whole approach to the question might have been different.

We may consider first the experiments in which cuts were made into the cotyledon from opposite sides, and then the tip alone illuminated.

When the cuts were not blocked in any way (loc. cit., p. 207) strong positive curvatures followed, but conduction may have taken place by diffusion straight across them. That they remained moist is suggested by p. 202.

Later, however, we read (p. 210) 'In a few (3) experiments, sheets of tin-foil were again placed in the cuts. . . . Nevertheless, a positive phototropic curvature followed, though it certainly remained very slight.'

The interpretation is difficult and further details are needed, especially as to whether, as a precaution against traumatotropism, the uppermost cut was made in half the cases on the far side from the light and in half on the near side. In my own attempts to repeat this experiment (admittedly with mica instead of tin-foil) no trace of a phototropic curvature could be found. However, the attempt was only made with a few plants, since, even with a single mica slip on the far side, the phototropic curvatures, if any, were so slight that there seemed no hope of obtaining a positive result with slips on both sides. Indeed, *Avena*, with its very weak near-side conduction, must be a most unsuitable plant for the purpose.



FIGS. 3-6.

In certain other experiments of Fitting the plants were split longitudinally, and one half removed. Of the possible arrangements, four concern us first. The split may extend to the responding zone or be limited to the tip. In either case, the remaining half may be on the far side from the light or on the near side (Figs. 3–6).

In the cases shown in Figs. 3, 4, and 5, Fitting, working with numerous plants, obtained positive phototropic curvatures. This result I have also obtained in 3 and 4, though on a small scale only. In the case shown in Fig. 6, Fitting obtained a positive result, but only when, after the split, the far-side half was shaded instead of being removed (loc. cit., pp. 216–17). He also obtained curvatures when the remaining halves were oriented sideways.

He concluded that these results were inconsistent with the 'straight-line' theory of conduction, and this conclusion seems to have become more or less accepted (cf. Stark and Drechsel, 1922, p. 368). But it does not at all necessarily follow. For on the 'straight-line' hypothesis the illumination of the intact tip (which is partially transparent) sets up a difference between its two sides such that they transmit back along the near side of the lower zones a feeble excitation leading to relative contraction, and along the far side a much stronger excitation leading to relative expansion. It is natural, then, to suppose that in the case of a half-tip a similar difference will be set up between its near and far sides, and lead to the transmission back in straight lines of corresponding excitations. If the consequences of this are considered for all the experiments with longitudinal splits, it will be seen that the phototropic curvatures obtained were really what was to be expected. Only in the case of Fig. 6 should we expect a doubtful result, for here a positive curvature could only be produced by the feeble 'near-side' excitation, while the stronger 'far-side' excitation would pass down the median plane of the stump, and so be ineffective. And it is exactly in this case that positive curvature is actually less easily obtained.

Indeed, these results only conflict with those unduly simplified views of straight-line conduction that relate the curvatures to the effect of the light on one side of the tip alone, and the excitation transmitted back along that side. But the possibility of conduction along either side of the cotyledon compels us to consider the resultant transmitted excitations as a function of the *difference* between the primary excitations set up by light on the two sides of the tip (see below): as soon as we do this, the supposed difficulties disappear.

The same consideration applies to Fitting's further experiment, in which a half-tip was illuminated from opposite sides equally, and no significant curvatures followed in the intact lower region. Since no difference would be set up between the two sides of the perceptive half-tip, one can understand that the conditions for the transmission back of excitation might not be present.

By the above, it is not in the least meant that we must ascribe to the plant any mysterious power of knowing that its two sides are unequally stimulated. It is only meant that, in tracing back the conducted excitations to the conditions at the tip, we must consider not only the direct effects produced by light on the two sides of the tip independently, but also the interactions between them. And this is clearly necessary, since firstly the fraction of the light that penetrates to the far side of the partially transparent tip must be quite large (the exact distribution of light within the *Avena* tip has not yet been determined), and secondly, the intensity of the stimulating light that will lead to positive curvature can be varied within a very wide range. From this it is clear that, independently (within limits) of the absolute intensities of illumination within the two sides of the perceptive organ, the 'near-side' excitation, leading to relative contraction, starts from the more intensely illuminated (near) side of the tip, while the 'far-side' excitation, leading to relative expansion, starts from the less intensely illuminated.

In this way of putting the matter, it is assumed that the effective factor in perception of light is the intensity difference between the two sides and not the direction of the light rays within the tissue. Should the latter view be adopted, it would be necessary to state the above argument rather differently. But the question of 'intensity difference' or 'direction of light-rays' is really one that concerns perception only, and is independent of the problem of the nature of conduction.

From the 'intensity-difference' point of view, it is a possible suggestion that there may be involved a transference of some soluble substance excreted by the more stimulated (near) side of the tip, and taken up by the less stimulated side. Experiments were carried out to test for the occurrence of such a process, but the results have so far been negative.

With regard to the claim of Van der Wolk (1911) to have obtained phototropic curvature in *Avena* after making blocked incisions from opposite side, his method is obviously open to error, since the cuts only reached just to the included leaves, without penetrating them (cf. criticism by Boysen-Jensen, 1913). It does not appear that there is at present any fully convincing evidence for the possibility of 'polarized' conduction, though naturally such evidence might at any time be found.

Finally, in connexion with the function of the tip of the grass cotyledon in phototropism, the interesting experiments of Bose on *Setaria* (1919, p. 362) should be noticed. His interpretation meets, however, with several difficulties. It is clear that the 'near-side' and 'far-side' excitations in *Avena* and the bean root have some resemblance to the two transmitted effects of excitation, of opposite sign, in terms of which he seeks to explain many of the phenomena of plant response.

SUMMARY.

1. In roots of *Vicia Faba*, decapitated and then reconstituted by replacement of the tip with gelatin, traumatic excitation can pass from the tip through the layer of gelatin and produce a negative curvature in the stump.

2. Tropic excitation due to traumatic stimulus or stimulus of gravity can be conducted back along either side of the root alone. A quantitative comparison is given of the efficiencies of the two sides in conduction, and in both cases a significant difference is found in favour of the side that afterwards becomes concave.

3. In the cotyledon of *Avena*, if the tip is removed and replaced in an anomalous position so that its far side (with reference to the light) is in connexion with the near side of the stump, then, on illumination of the tip alone, there follows phototropic curvature *away* from the light. It is clear that the

excitation transmitted from the far side of the tip simply causes relative expansion in that side of the lower region (whichever it may be) down which it passes.

4. The excitation conducted down the far side of the *Avena* cotyledon can cross a gap filled with moisture.

5. Reference is made to certain experiments by Fitting from which he concluded that phototropic excitation in *Avena* can be conducted by a sinuous path and must determine the direction of curvature in virtue of some 'polarity' possessed by it. Reasons are given for considering that his experiments do not necessitate these conclusions.

THE DEPARTMENT OF BOTANY, OXFORD,
May, 1923.

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A Suggested Explanation of the Mechanical Action of Lithophytic Lichens on Rocks (Shale).

BY

E. JENNIE FRY, M.Sc.,

Assistant Lecturer in Botany, University College of Wales, Aberystwyth.

With ten Figures in the Text.

IT is well known that gelatin when drying will tear up the surface of a glass plate. Ostwald and Fischer, in their book 'Practical and Applied Colloid Chemistry', state, for example, 'When a gelatin solution is dried upon a glass plate, as in an oven at 100° C., the gel contracts. But at the same time it sticks so fast to the glass that large shell-shaped pieces are torn off the surface of the glass.'

It is well known that lichen thalli contain a large proportion of gelatinous or mucilaginous substance, and in view of this fact it seemed possible that a phenomenon, similar to the above, might be the explanation of the 'mechanical force' exerted by lichens in bringing about the disintegration of the surface layers of rock on which they grow. A review of published work on lichens and lichen ecology has brought out the fact that, although the 'mechanical force' exerted by lichens in the disintegration of rock is usually given some amount of prominence, nowhere in these publications is there an attempt to explain the nature of the initial force.

SECTION I.

Experiments with Gelatin.

Lithophytic lichens, particularly thalloid and crustaceous forms, are exposed to periods of extreme drought—caused by drying winds and also by the high temperatures which the surface layers of rocks attain when exposed to the full glare of the sun. It seemed possible that the drying of the more or less gelatinous lichen tissue might result in a breaking up of the rock surface, similar to that obtained when gelatin is dried on glass at

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100° C. In order that such a comparison might be drawn between the two, it was necessary to experiment with gelatin drying on glass at temperatures within biological range.

Jumelle's experiments (1) give some idea of the high temperatures endured by these plants. He found that respiration of certain forms was practically normal after being exposed for three days to a temperature of 45° C., fifteen hours at 50° C., and then five hours at 60° C., but as a rule carbon assimilation ceased after the plants had been kept for twenty-four hours at 45° C., three hours at 50° C., and then half an hour at 60° C. Also Zopf (1) stated that on a hot June day a temperature of 55° C. was endured by crustaceous lichen thalli. Considering the above statements, it is reasonable to assume that, during the middle hours of the day in summer, a temperature of 45° to 50° C. obtains in lithophytic lichen thalli, and that the conditions to which drying gelatin would be subjected at such a temperature would be similar to those experienced by such a vegetation.

(a) *Experiments with Gelatin on Glass.*

Aqueous gels of gelatin of concentrations 2·5, 5, 10, and 20 per cent. were used, but 20 per cent. was found generally to be the most effective. A small amount of the gel was allowed to set on the glass surface, and the slide then placed in an air oven, the temperature of which was known. The slide was withdrawn after a certain period of time, and the gelatin surface examined. After the gelatin had been removed by dissolving in hot water, the glass surface immediately below was examined.

Temperature of oven.	*Period of time.*
(*a*) 15° C., gradually rising to 100° C., where it was kept constant for a few minutes . .	about ½ hour
100° C.	,, 1 hour
(*b*) 60° C.	,, 2 hours
(*c*) 40°–48° C.	,, 2 hours
(*d*) 30°–50° C.	,, 3 hours

After withdrawal from the oven, in each case the gelatin film was cracked or chinked—indicating the contraction of the gel. In many cases the dried layer had been lifted from the glass below. Some of these pieces were removed and the lower sides were seen to be encrusted with small flakes or scales of glass, each showing conchoidal fracturing. The glass slides used were freed of gelatin and the exposed glass surface showed the following features:

(*a*) The smooth polished surface had been destroyed, particularly in those parts below the peripheral regions of the gelatin film. The surface appeared as if very finely chipped or etched.

(*b*) As in (*a*), but the chipping was much more distinct—the plates removed from the surface being much larger than when gelatin was dried at 100° C. Often the chipped regions appeared to radiate inwards from the peripheral margin, and in a number of cases extended as far as the centre of the area of glass previously covered by gelatin.

(*c*) and (*d*) These appeared very much as (*a*) and (*b*), but the chipped areas were larger and fewer.

A few experiments with gelatin drying on glass under room conditions were made from May 8 to 11, during which time there was a fair amount of sunshine. The gelatin cracked and lifted from the surface in several places, and flakes of glass, somewhat larger than those detached at 60°, were chipped off the surface by the mechanical action of the contracting gelatin.

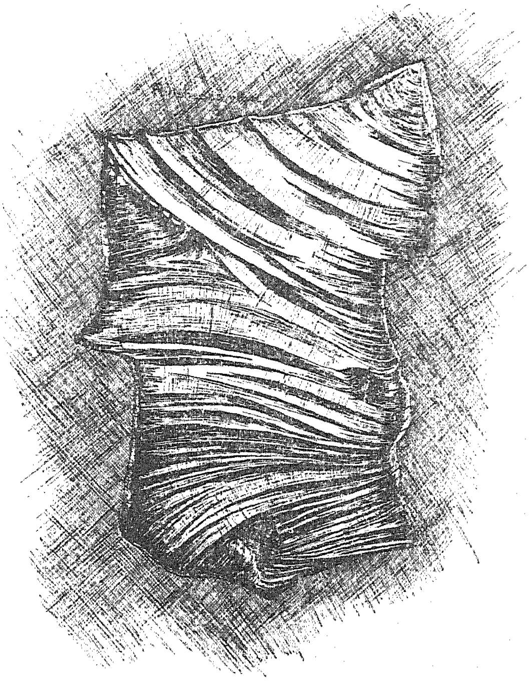


FIG. 1. Glass surface showing small chipped area. Appearance due to drying gelatin. (See text.) Note striations and conchoidal fracturing.

CONCLUSIONS.

Gelatin drying on the surface of glass below 100° C. is able to chip off the surface layers and to lift off from the surface the scales or flakes thus detached.

This action goes on at temperatures within biological range. The higher the temperature the smaller the flakes chipped off by the gelatin, and the greatest effect is obtained below the marginal regions of the gelatin film.

By covering numerous small areas instead of one large one, in order to increase the circumference in proportion to the area, it was thought that greater destruction of the surface might be effected. However, it was found that this was not the case. This was probably due to the fact that the thickness of a gelatin film over a smaller area was less than that over a large one, and that the contracting forces operating in the former case were not sufficiently powerful to bring about the same results as in the latter. This was proved by the fact that when the thickness of the gelatin over the small areas was increased by adding successive layers of the gel, and then dried, the surface of the glass was chipped to a considerable extent. By repetition of this process many times on the same area of glass, the level of the surface was lowered. As the glass became more and more roughened by the constant drying of fresh gelatin films so the rate of 'burrowing' became greater. This was particularly the case once a small, more or less circular pit or depression had been formed. The drying gel lifted the glass fairly readily at the sides of the floor of the depression, but less readily in the central regions. This was indicated by the touch and also by the fact that the first appearance of a hole through the glass was round the edges of the base of the pit. Finally, the central thicker portion was separated from the slide, leaving a circular hole. Several perforations of glass have been made in this way. The shortest time taken to bore a hole through a glass slide of 1 mm. thickness was ninety-six hours. This was done by working with 20 per cent. gelatin, and drying at 60° C. After eleven or twelve applications of gelatin the slide was completely perforated. In these experiments, if the detached glass flakes were not removed from the surface of the small depressions, a peculiar iridescence was noticed in the glass in these regions.

(b) *Experiments with Gelatin on Shale.*

Having established the fact that the surface of polished glass is disintegrated by the drying of gelatin at temperatures within those experienced by lichen thalli, experiments of a similar nature were tried upon *unweathered* smooth pieces of shale. The object of these experiments was to ascertain whether the mechanical force could operate in a similar manner on this rock, which does not possess the highly polished surface of glass, and which, in addition, has a certain porosity of texture. Although the rock was treated in exactly the same way as the glass, preliminary experiments with the shales showed that the action, besides taking a longer time, was of a rather different nature. This latter was probably due to the different texture of the shale. For example:

1st drying	40° to 80° C. for 2 hours.
2nd ,,	40° to 55° C. for $1\frac{1}{2}$,,
3rd ,,	30° to 50° C. for $1\frac{1}{2}$,,

After the first drying no change was noticed. The shale was allowed to cool under the atmospheric conditions of the laboratory and then replaced in the oven. At the end of the second period, the edge of the gelatin film was noticed to be slightly raised from the surface of the rock, and on the under side of this separated film there appeared to be attached a continuous film or plate of shale. After cooling, the shale was again replaced in the air oven, and after the period quoted the marginal regions of the gelatin layer were distinctly raised up from the surface of the substratum together with a continuous layer of shale. These parts of the gelatin film with their attendant shale plates were easily detached from the surface of the rock, and in the latter, where the gelatin had been, slight depressions were visible. These depressions coincided exactly with the outline of the gelatin films. The surface of the shale had been literally peeled off.

As in the case of the glass, the marginal region being the seat of greatest activity, numerous smaller areas were covered with gelatin rather than a single large one.

1st drying	40° to 55° C. for $1\frac{1}{2}$ hours.
2nd ,,	30° to 50° C. for 3 ,,
3rd ,,	30° to 45° C. for 3 ,,
4th ,,	40° to 50° C. for 3 ,,

Between each drying the shale was cooled to room temperature under laboratory conditions.

After the fourth drying period the margins of the larger films, with shale flakes adhering, were curled upwards away from the rock surface, but the central part of the films remained attached to the substratum. These films were easily removed from the surface by gently pulling with a pair of forceps, and small depressions remained in the substratum. These depressions coincided exactly with the position of the gelatin films. It was found that the thickness of shale attached to those films was greatest in the marginal regions and least in the central part, where the film had remained attached to the rock. In fact in this central part the shale was represented merely by a film of more or less isolated mineral fragments. The smaller areas of gelatin were not affected after the four drying periods; but in experiments with longer times for drying the gelatin, even they were affected in a manner similar to that described above, except that the detached shale flakes were very thin. Even this only took place when the gelatin film was fairly thick:

1st drying . .	44° to 56° C.	for	4 hours.
2nd „ . .	46° C.	„	6½ „
3rd „ . .	49° C.	„	2 „
4th „ . .	40° to 56° C.	„	5 „
5th „ . .	30° to 56° C.	„	2 „

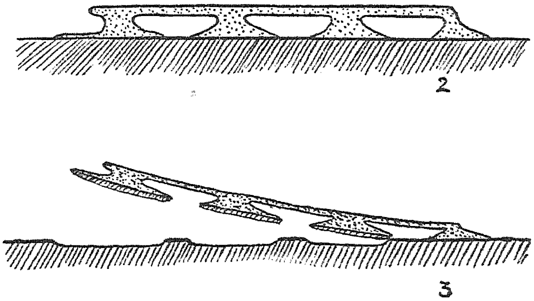
After the first period of drying, the peripheral regions of gelatin films of diameter 5 mm. and over were raised very slightly from the surface, together with the film of shale. Following the second, the separation of these films was quite distinct, and also the very small ones of diameter 1·5 to 1 mm. were beginning to show a lifting up at the margins. This was quite pronounced after the third period.

After continuing the drying, the gelatin patches with their attached shale plates were easily removed from the surface by gentle brushing, or pulling with a pair of forceps. As before, small depressions of the exact size and shape of the gelatin films were left in the surface layers of rock.

It was commonly noticed that where the marginal regions of gelatin had not been raised by repeated drying the gelatin became cracked or 'chinked', and that the gelatin bordering these chinks curled upwards away from the rock below, bringing with it on its lower surface shale films or plates similar to those pulled from the rock in the marginal regions of the other examples quoted. Also where the marginal regions were very thin and apparently unable to tear away the shale, cracks or chinks appeared in the thicker gelatin close behind this thin zone. At this point the film became raised from the substratum as described above. It was in this way that the thin marginal zones of a number of these areas eventually were lifted from the shale surface, but the shale attached to their lower surfaces was the merest film of mineral particles. This chinking of the gelatin films has been observed when the latter have been drying in the sun in the window during May.

The phenomenon reminds one very strongly of the 'chinking' of crustaceous lichens, and in this connexion it may be worth remembering that each separated portion of the gelatin—separated from the rest by cracks—is able to lift off from the substratum films of shale. The present discussion deals with foliaceous thalli only, but in a later paper it is hoped to give details of the mode of attack on rocks of crustaceous lichens.

When the gelatin films of small area were made of greater thickness in the centre and these thickened portions made to fuse on to a continuous plate of gelatin of the same consistence, then, on drying the whole for two or three days, even under room conditions during May, it was found that the discs of gelatin—covered on their lower surfaces with shale—were torn from the substratum. The continuous plate of gelatin, on drying, curled upwards away from the substratum, and it was due to the contraction of this layer—together with the adhesive power of the contracting gelatin of the discs themselves—that the shale surface was torn off in the regions immediately below these gelatin films (Figs. 2 and 3). This method of removing the surface of the shale takes much less time than when working only with the gelatin films of small size, and it is to the continuous layer of gelatin attached to the discs that this greater activity is to be attributed. The gelatin mass in these experiments was meant to represent the form of foliaceous thalli.



FIGS. 2 and 3. Diagrammatic vertical section of gelatin and shale. Fig. 2, before drying; Fig. 3, after drying. Dotted area, gelatin; shaded portion, shale.

SECTION II.

Lithophytic Lichens on Shale.

It has been proved that when films of gelatin on unweathered shale are dried at temperatures within the range of those experienced by lithophytic lichen thalli, owing to the contraction experienced by the gel on withdrawal of water, the superficial layers of rock are peeled off, leaving exposed fresh layers of the substratum. It remained to be shown whether lichens growing on this substratum and exposed to similar conditions would exhibit the same remarkable mode of action.

Choice of Material.

Of the lithophytic foliaceous lichens in the Aberystwyth district, perhaps the most common and typical is *Xanthoria parietina*; consequently this species was chosen to represent the class.

Fresh specimens of *Xanthoria* colonies of different sizes were obtained *in situ* on the shale. As far as possible only those were chosen which grew directly on unweathered and unaltered shale, and not those which succeeded a pioneer crustaceous vegetation. The central portions of very many of the larger or older colonies were absent—the bare rock being exposed in these regions. Commonly these exposed surfaces of the shale were in various stages of recolonization by young thalli of *Xanthoria* or other foliaceous species, such as *Physcia stellaris*, so that these latter were surrounded by the complete growing marginal zone of the older colony.

This wearing away of the older parts of *Xanthoria parietina* has been attributed to the action of blown sand (2), *Xanthoria* being particularly sensitive to this action. But the same appearance has been noticed by the writer in this and in other species, where there could be no sand action. For example, certain crustaceous and placodioid *Lecanora* species, growing on inland limestones and shales, frequently have their central regions worn away. *Lecanora murorum* on the Anglesey carboniferous limestones affords a particularly good example of this phenomenon, the thallus being frequently represented by five or six, or more, growing marginal regions, almost concentrically arranged. Reference to this state will be made again.

Xanthoria Thallus.

It may be as well, before describing the effect of drying experiments on *Xanthoria parietina*, to recall the structure of its thallus. For this purpose a vertical section will suffice (Fig. 4). There are two cortices—upper and lower—which are composed of closely interwoven hyphae forming a pseudoparenchymatous or plectenchymatous tissue. The walls of these hyphal cells are very swollen and gelatinous, and by coalescence a continuous tissue is formed. Limiting the upper cortex to the outside is a very thin, structureless, yellowish-brown zone—probably the main accumulation of parietin. Immediately below the upper cortex comes the gonidial layer, and between the latter and the lower cortex is the medullary zone.

In exposed positions the bright orange thallus appears to be pressed very closely to the rock surface, but as a matter of fact it is only attached thereto by short outgrowths from the lower side of the thallus. The outgrowths or haptera, which arise closely behind the growing margin, appear first as small white projections formed by outgrowing cortical hyphae. Where the haptera come in contact with the substratum, the hyphae forming them spread out in a radiating fashion over the rock, and give rise to discs or plates of tissue. The size of these discs varies considerably, but commonly a diameter of 1 mm.–1·5 mm. is reached (Fig. 5). The cells of the hyphae forming these organs are more elongated than those of the cortical tissue from which they arise. In the stalks they are arranged parallel with each other, forming dense structures, from the base of which the hyphae of the discs radiate. The central region of a single attachment plate is thicker than the marginal zone, which is composed of fewer and fewer hyphae as the extreme periphery is approached. The writer has found, in every case examined, that there is a peripheral zone of mucilaginous or gelatinous substance, of an average width of about 5 μ, extending beyond the limits of the radiating hyphae (see Figs. 5 and 6). The origin of this gelatinous substance is not known at present, but since the material of a similar nature in the cortices is obviously derived from the cell walls of the hyphae forming that tissue, so it may be that the gelatinous zone of the attachment disc arises by alteration of the walls of the hyphae forming that organ. Additional haptera are frequently formed where the tip of the growing margin touches the substratum. Hyphae from the lower cortex grow out and beome attached to the rock. Ultimately a hapterum, similar to those which arise normally, is formed just under the very margin of the thallus lobe (see Fig. 7, A).

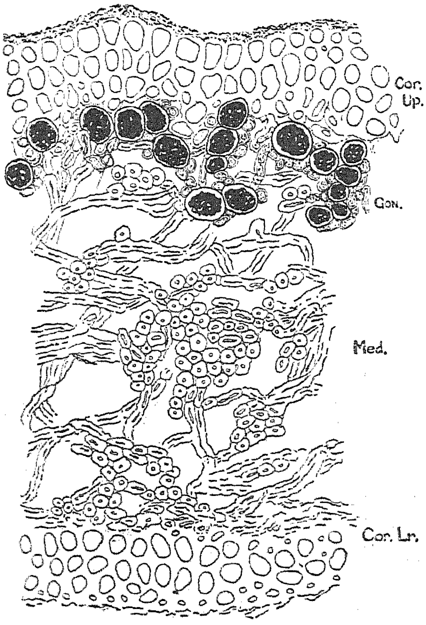


FIG. 4. *Xanthoria parietina.* Vertical section of thallus. *Cor. Up.* and *Lr.*, upper and lower cortex; *Gon.*, gonidial layer *Med.*, medullary layer.

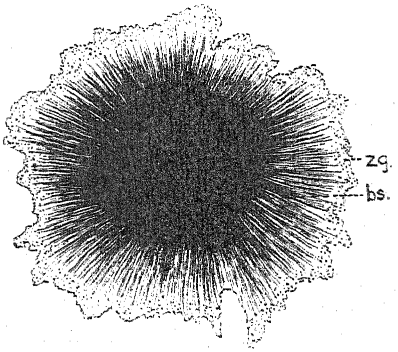


FIG. 5. *Xanthoria parietina.* Diagram of hapteral disc. *zg.*, zone of gelatinous substance; *hs.*, base of hapteral stalk or stipe.

Drying Experiments with Xanthoria parietina 'in situ' on Shale.

The same procedure as that adopted in the experiments of drying gelatin on glass and shale was followed when dealing with lichen thallus on shale.

Old and young colonies of *Xanthoria* were placed in the drying oven at known temperature, and after a certain period of time were withdrawn, examined immediately, and allowed to cool under room conditions. In some experiments the thalli were moistened by gently brushing with a wet brush. After cooling—usually overnight—they were replaced in the oven and the process repeated. After each period of drying the state of the thalli was noted.

Generally speaking, those colonies of *Xanthoria* of the same age and collected from exactly similar habitats, when subjected to the same drying conditions, responded in a similar manner. Colonies chosen indiscriminately, i.e. some from very exposed lamination planes, others from the exposed edges of the beds, and others from shaded or damp parts of the rock, differed from each other in the amount of response. Consequently, although temperatures and times are given in the experiments cited below, these need not indicate the exact temperature or period of time at which certain results would be obtained with any thallus. Because of this fact, although many experiments were carried out, the exact details of only two series are given below. Of the rest a general account of observations will serve.

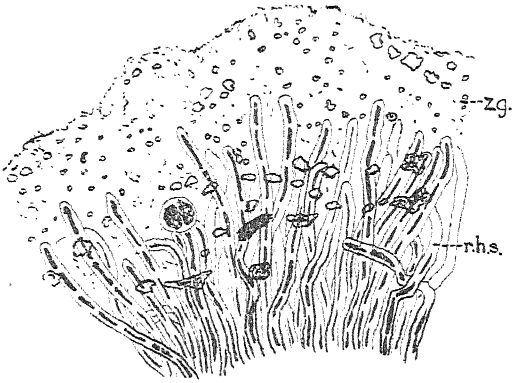


FIG. 6. *Xanthoria parietina.* Part of marginal region of hapteral disc, showing fragments of shale. (Lifted from shale when wet.) *zg.*, zone of gelatinous substance; *r.h.s.*, radiating hyphae from hapteral stalk.

OBSERVATIONS.

Time.	*Temp.*	*Thalli of moderate age.*	*Old thalli.*
1½ hrs.	32°–46° C.	Thalli very dry and hard, but brittle. Tips of marginal lobes curled slightly upwards away from rock.	Ditto.
8½ hrs.	30°–56° C. (mainly 38°, but sudden rise at end of period).	The same as above, but more pronounced.	Where colonies had been divided, older parts of thalli were lifted up from the rock and curled upwards. Haptera had shale plates or fragments attached to basal discs. The surface of the shale below appeared to be weathered and showed many small depressions. The surface had been removed in the position where the haptera had been attached.

Time.	*Temp.*	*Thalli of moderate age.*	*Old thalli.*
6¼ hrs.	46° C.	As above.	As above.
2 hrs.	49° C.	Central parts of colonies broken from substratum. Parts of marginal zone curled back, with an occasional hapterum detached from the rock.	As above.
1¾ hrs.	45°–56° C.	Where upper lobes were fixed to lower by haptera or by fusion of the tissues of the thallus proper, strains were set up between the overlapping lobes. Lower thallus, particularly the upper cortical tissue, was frequently torn by such strains; and occasionally whole apothecia were pulled off from lower thallus by the haptera of the upper. Marginal regions were curled well back, but very few haptera in these parts were detached. Very great difference in the number of detached haptera in old and young parts of the same thallus.	Older parts were raised well above the surface, showing large numbers of detached haptera.
5 hrs.	40°–56° C.	Where crustaceous thalli were succeeded by *Xanthoria*, parts of the former were torn up by the marginal haptera of *Xanthoria*. *Lobes of the Xanthoria* were torn apart, showing large gaps in the colonies, particularly in the older parts. (Later there appeared tears across the lobes, separating the younger from the older portions.) On moistening, the gaps readily closed up again, the thallus appearing quite normal and sinking back into its original position.	On moistening, the thallus appeared to revive and soon assumed its normal position against the rock surface.

General Account of Experiments.

(a) *Young Thalli.*

The curling up of the lichen seemed to be due to an early contraction of the upper side of the thallus, which was in contact with the dry atmosphere. It was an exactly similar effect to that produced by the drying gelatin 'thalli' on shale. The air below the thalli gradually became less moist and water was withdrawn from the lower side, which in consequence contracted. This limited to a certain extent the amount of curling upwards of the lichen. Apart from this, the amount of curving upwards of the thallus depended on: (1) the proximity of the haptera to the edge of the lobes, and (2) the nature of the substratum—that is to say, whether the thalli were fixed on to the unweathered rock, to weathered shale, or crustaceous lichens. In some cases the haptera were developed some distance behind the growing margin, and in these there was a considerable curl on drying (Fig. 8, A, B, C). In others, as previously stated, the haptera were situated at the extreme margin, and here the curling back was restricted until the haptera were torn free from the substratum (Fig. 7, A, B, C). It was evident in both cases that, owing to contraction of the thallus, great strains were set up, both in the lichen tissues and in the substratum. The thalli seemed to recede by a kind of shearing movement. This was particularly evident in the case of those lobes with marginal haptera. These were left exposed beyond the edge of the thallus (Fig. 7, B). On moistening the plant, the haptera were again hidden by the swelling of the lichen tissues (Fig. 7, A). Where the substratum was of weathered rock or a crustaceous lichen the curling of *Xanthoria* was more marked, for the simple reason that it was easier for the latter to tear away fragments of those substrata than it was for it to pull off the surface layers of unaltered rock.

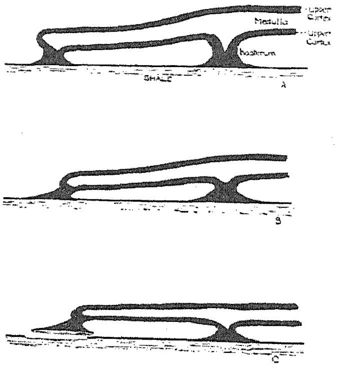


FIG. 7. *Xanthoria parietina.* Thallus with marginal haptera. A, moist condition; B. C, stages of drying.

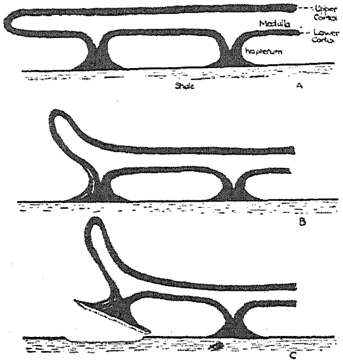


FIG. 8. *Xanthoria parietina.* Thallus with haptera at some distance from the margin. A, moist condition; B, C, stages of drying.

The hapteral discs remained so firmly attached to the substratum that when the thallus contracted either:

1. The substratum attached to the disc came away with the hapterum, as in the case of the crustaceous thalli or weathered rock;

2. The haptera pulled away a very thin film of rock, as was the case occasionally on unweathered shale; or

3. The haptera remained so firmly attached that the thallus, being unable to withstand the enormous contractile strain, tore across at right angles to the direction of this tension.

To imitate the action of lichens on shale and crustaceous lichen substrata, experiments with artificial thalli—made of gelatin—were carried out (see Section I, (b)).

The tenacity of the hapteral discs has led one to compare them with the small thin areas of gelatin on the shale. In both cases, when dry, they were extremely difficult to remove. Often, when young parts of *Xanthoria* were pulled, the thallus tore free of the haptera, leaving the latter standing on the rock. When such were dried, like the small areas of gelatin, there was great difficulty in getting them to separate from the substratum.

On moistening, both gelatin films and the discs rapidly became soft and were easily detached. They absorbed water at about the same rate. The haptera, which before were hard, brittle, and white, in a few seconds became soft, swollen, and translucent. It is owing to the ready absorption of water by the hapteral discs that an easy removal of the thallus as a whole can be effected, so long as the lichen is saturated with water.

(b) *Older Parts of Thalli.*

Not only did the marginal lobes curl away from the substratum on drying, but the older parts of the thallus responded in the same way. In these regions many haptera, with plates of shale attached to their basal discs, were separated from the substratum, owing to the contraction of the thallus. The number of detached haptera in the older parts was far greater than in the young growing regions. Apart from any chemical change which may have caused a softening of the superficial shale below the older parts of the thalli, the greater effect in these regions was probably the result of the continual strains set up in the substratum during the growth of the lichen. Owing to alternation of dry and wet periods, these strains would occur at intervals during the development of the thallus, and ultimately planes of weakness would be formed between the top lamina and the one immediately below, in the parts of the substratum where the haptera were attached. This condition being reached, the contracting thallus would cause the separation at the planes of weakness of these superficial plates of shale.

It should be noticed that in the case of the artificial thalli the forces at work were sufficiently great to bring about this result at one contraction, whereas in the case of the lichen thallus it probably took many contractions, for it was only in the older parts of the colonies that this separation readily took place—though it did occur occasionally in the marginal regions. In the older parts of the thallus growth of the tissues had probably ceased, and then, when a plate of substratum was detached, the thallus was incapable of gaining a fresh hold. In the young parts this was possible by the growth of hyphae round the detached fragments, followed by the re-attachment of these new outgrowths from the hapterum to the substratum immediately below. In this connexion one may mention the refixing of young plants of *Physcia stellaris* to a shale substratum. Young thalli were detached from the rock and then placed on shale in a moist atmosphere. It was some weeks before a firm attachment was effected, and when this had taken place the thalli were detached again from the substratum and the hapteral discs examined. To the mats of young colourless hyphae arising from the end of the dark brown haptera were attached small particles of shale which had been torn from their position when the thalli were detached from the rock. One concludes that this is what happens under natural conditions when the thalli contract: particles are detached, enclosed, and a fresh hold taken.

That this actually does take place has been shown by the incorporation in the hapteral disc tissue of plates of shale, and when *Xanthoria* grows on trees, by the similar incorporation of pieces of bark. Miss Mellor (3) found flakes of glass in the haptera of *Xanthoria* when the latter grew on glass. The presence of the glass in that position may be attributed to a similar action.

It was in the attempt to imitate such action of the growing hyphae of the lichen enclosing separated flakes of substratum that the holes were bored though the glass (Section I). Since the gelatin did not possess the property of growth and of surrounding the detached scales of glass, fresh applications of gelatin were made on the place from which the glass had been torn. The fresh gelatin represented the new outgrowths of the haptera enclosing the fragments and obtaining a hold immediately below.

Miss Mellor (3) stated that M. Felix Gaudin had found pieces of glass perforated by the action of lichens. The present writer attributes this perforation mainly to the mechanical action of the lichens, which can be imitated by the drying of gelatin on a substratum of glass (see Section I).

When the older parts of the thallus had been raised from the substratum, the latter, as previously mentioned, was seen to be 'pitted'. This was due to the tearing away of the plates of shale by the hapteral discs, for the position and size of the pits corresponded with the position and size of the detached haptera on the raised thallus. It was a similar effect to that obtained when gelatin 'thalli' were dried on a similar substratum (Figs. 2 and 3). After washing and gently brushing the surface, these pits were still found to exist. This was unlike the result obtained by Miss Mellor (3), who, after treating in a manner similar to that described above the slate surface below foliaceous lichen thalli, found that the pits disappeared and the surface showed no sign of the former epilithic vegetation.

The floor of the depressions was always of dark grey, unaltered shale, but the general surface of the substratum appeared, as a rule, lighter in colour. This is probably to be explained by the chemical action of the carbon dioxide of lichen respiration on the rock surface uncovered by the hapteral discs.

In the drying experiments the ready separation of the older parts of *Xanthoria* colonies can be explained. But practically the same conditions are repeated in nature, and consequently the same effect is produced. The older parts of the thalli, raised from the substratum, become so brittle and dry that a strong wind is sufficient to remove them, or, where they occur on vertical surfaces, their own weight causes the removal. The presence of cracks across the thallus, caused by the strains set up in the tissues, also contributes towards the separation from the substratum of the older parts of the lichen. Besides the more obvious lateral separation and tearing apart of the lobes of the lichen, many of the experiments showed this cracking and splitting across at right angles to the long axis of the thallus.

It may be that blown sand helps in the final removal of the parts of *Xanthoria* colonies detached from the substratum, when the lichen grows in a position where such an action could take place, but it does not seem to be the only explanation for such removal. Other lichens, even crustaceous forms, suffer the crumbling away of their thalli when not exposed to the action of blown sand.

(c) *Expansion and Contraction of Xanthoria Thallus.*

To measure the expansion and contraction of *Xanthoria* caused by the addition and withdrawal of water from the tissues, thalli were dried at different temperatures, and strips from the young growing zone were cut off parallel with the long axes of the lobes. These strips were mounted dry on a slide, the length and width measured by means of the micrometer. Water was then introduced under the cover-slip and time allowed for the tissues to absorb the moisture. The strips of thallus were then remeasured. The difference between the two sets of measurements gave the amount of expansion of which the thallus was capable. It also indicated the amount of contraction which could be brought about by drying the lichen :

State of material.	*Time and temperature of drying.*	*Length expansion.*	*Width expansion.*	*Difference in the two expansions.*
Undried material :				
Fresh collected on dull day		%	%	%
(*a*) Young edge	—	2	6	4
(*b*) Older part	—	8	10	2
Room-dried material	Room conditions	14	20	6
Oven dried (young)	2 hrs. ; 32°–36° C.	9	14	5
Oven dried (young)	$\frac{3}{4}$ hr.; 20°–60° C.	8	11	3
Oven dried (young)	2 hrs. ; 40°–60° C.	11·5	14	2·5
Oven dried (dead)	$1\frac{1}{2}$ hrs. ; 80°–96° C.	22	25	3

Each of the figures given above was obtained by taking an average of the expansions of a number of experiments.

There was a considerable variation in the results obtained, but the average percentage brings out very clearly the fact that the tangential expansion or contraction was always considerably greater than the radial. This supports the observations made on drying complete colonies of *Xanthoria*. The lobes of the thallus separated from each other, leaving large gaps, exposing the substratum, between them. It was only later that there occurred the tearing across the lobe at right angles to the long axis of the thallus. The fact that it has been proved by measurement that the tangential expansion, or contraction, is greater than the radial supports the theory that the effect of lichens growing on rocks is similar to the action of drying gelatin on glass or rock. The first and more obvious cracks or chinks which appeared in the drying gelatin were, more often than not, radial. This indicated a tangential contraction and caused the areas of chipped glass to be mainly in radiating bands as stated (p. 177).

To indicate further the swelling capacity that this lichen possesses, it may be stated that, on addition of water to vertical sections of the dry thallus, they expanded 38 per cent. of the original depth. (This figure again represents an average expansion.)

SECTION III.

Discussion of Points from Previous Work relating to the Present Investigation.

Although the present writer does not entirely agree with Miss Mellor (3) in her explanation of the deterioration of the glass surface by lichens, the phenomena described by the latter seem to give strong support to the explanation here suggested:—

'Deterioration of the Glass: Both surfaces of the glass are corroded, showing opaque glass in the form of circular patches, glass which has become squamose and iridescent, as well as holes covered with scales of glass and bordered by opaque glass. The round patches of glass run together sometimes in consequence of their increase in size. The central part of the patch disappears and one can distinguish a circular hole which gets larger as the patch of opaque glass gets smaller. These primary holes run together and give rise to larger holes of irregular shape, always bordered by opaque glass—the width of the border varying. In my specimens the corrosion attains a maximum depth of 1·6 mm. and a width of 5 mm. M. Felix Gaudin has seen glass perforated by the joining up of holes from either surface. . . .'

Examination of the fragments from the glass surface shows that there are in them holes and striations. These striations are in the surface of the glass and 'appear sometimes as scales piled on top of one another, sometimes like ridges made in the sand by the waves' (see Fig. 1). Gentle pressure causes the fragments to 'break along the axis of the striations and there then appear the small superimposed scales'. (This is the *squamose glass* mentioned.) Miss Mellor then gives an outline of the researches of Moissan (4) and Germann (5), showing that by the action of carbon dioxide and water the surface of glass becomes altered chemically: 'The silicates of the glass are more or less hydrolysed, with the formation of silicic acid and calcium and sodium hydroxides. The bases absorb carbon dioxide from the air and form the acid carbonates of calcium and sodium, and these are dissolved and removed. When the lichens grow on church glass the chemical action is accelerated, because rain-water and condensed water vapour are held there by capillarity and are charged, moreover, with carbon dioxide as a result of respiration of the lichens. This increase of chemical action is made evident by the fact that the glass attacked by the lichens becomes opaque or squamose and iridescent. *One notes this change over the whole extent of the attachment of a crustaceous lichen. The place occupied by a lobe of a foliaceous lichen is less opaque than that of its haptera.*'

The present writer does not attribute to the chemical action of carbon dioxide and water the state of the glass surface below the lichens, for if they were responsible there would be the same effect found below any lichen living in close contact with the glass, and this is not the case. Miss Mellor states that the surface of glass below *Lepraria flava*—a powdery lichen—remains without any trace of deterioration. There must be some other explanation of the deterioration of the glass surface—one that accounts for the corrosion below crustaceous and foliaceous species, and the absence of the corrosion below powdery forms, such as *L. flava*.

In very many crustaceous species examined by Miss Mellor glass flakes were found embedded in the thallus tissue, more particularly in the apothecial and spermogonial regions. In the thallus, and particularly in the specified regions of unusually vigorous growth, carbon dioxide is evolved, yet there appeared no chemical alteration of the glass, but merely separation of the flakes, the flakes showing the usual surface with striations and indentations (Fig. 1). This, one gathers, was still the case with the scales of glass left on the substratum after the thallus had decayed.

It has been shown (see Section I, (a)) that iridescence of the glass can be obtained by the non-removal of flakes of glass detached from the surface by the mechanical action of drying gelatin, the effect being due to discontinuity of the homogeneous substance along the lines of fracture. The surface of the glass below, and also that of the fragments themselves, when detached, showed indentations and striations. This appearance, together with iridescence, Miss Mellor attributes to the chemical action of carbon dioxide and water, but both have been shown, during the present investigation, to be produced by the mechanical action of gelatin drying on the surface of glass.

In the gelatin experiments the surface was corroded where the gelatin was attached to the substratum. So in the case of the lichens, the surface of the glass was rendered opaque or squamose and iridescent 'over the whole extent of the attachment of a crustaceous lichen, but the place occupied by a lobe of a foliaceous lichen is less affected than that of its haptera'; in fact it is stated that when *Xanthoria parietina* thalli were gently raised from the substratum, the haptera either remained firmly attached to the glass, or brought away with them small flakes of the substratum. If the action of the lichens on the glass were mainly chemical, it would seem that the surface of the glass below the lobes of a foliaceous thallus would be more open to chemical attack by the carbon dioxide of respiration than those particular areas of surface covered with the gelatinous discs of the haptera, since gaseous exchange through the gelatinous film of the dry hapteral discs would be practically nil, and even when the discs were moist the action of carbon dioxide on the substratum immediately below them would naturally be retarded. Yet it was in these positions, where the haptera of a foliaceous lichen were attached, that the action was the greatest.

Of the lichens growing on glass the crustaceous species predominated—in agreement, according to Miss Mellor, with their greater resistance to wind and rain. This greater resistance, the present writer considers, is due directly to the fact that the initial mechanical action on the glass of crustaceous forms is slower than that of foliaceous species. This is clearly brought out by comparing the mechanical action of simple thin films of gelatin with that of artificial gelatin thalli (Section I, (b)).

The presence of the gelatinous cortices of crustaceous and foliaceous lichens, together with the close adhesion of these forms to the substratum by gelatinous substance of the hypothalli and the hapteral discs respectively, is considered to account for the corrosion of the glass below these lichens, since the enormous contractive forces of the cortical gelatin would be able to operate on the surface of the substratum. Conversely, the absence of continuous gelatinous tissues in the powdery thallus of *Lepraria flava* would account for the absence of deterioration of the polished glass surface over which the lichen grows.

It would appear, then, that the chipped appearance of the glass, attributed by Miss Mellor to chemical action, is not chemical but mechanical, and dependent on the structure and nature of the thallus itself.

Once the initial mechanical disintegration of the surface has been accomplished, then the more obvious second stage of mechanical action is apparent. It is this 'mechanical action' which Miss Mellor describes as acting on the '*chemically decomposed*' glass :—

'Scales of glass like those found on the opaque and squamose glass windows were found embedded in the thalli of lichens.' As these thalli decomposed so the flakes or scales were liberated from inclusion in the tissues. On the surface over which lichens had been growing free scales were present, particularly where traces of former lichen inhabitants were seen. 'The indentations and striations of the decomposed glass resemble the primary and secondary cleavages in a rock, and the decomposed glass surface—like the chinks in a rock—offers a lessened resistance to a penetrating as compared with a pressing organ. It is on this "*decomposed*" glass that the vitricole lichens are most closely attached. The hyphae, applied to the "*decomposed*" glass, must exercise on it a pressure which varies according as they are, or are not, in a state of growth or turgescence. As the mechanical action operates the glass disintegrates, and in consequence its constituent parts, in small scales, are separated from one another. The hyphae, in growing, follow the planes of cleavage, and, moreover, on account of their pressure, the hyphae raise the scales of glass which are subsequently gradually incorporated in the tissue of the lichen. It is therefore by the mechanical action of the vitricole lichens that the breaking up of the disintegrated glass is to be explained, and the same action accounts for the small hollows on the surface of the piece of glass.'

This *mechanical* action of lichens can be made clearer by a comparison of the above with the action of a powdery lichen—*Lecidea lucida*—on shales. This species grows well on shales which dip at a low angle with the outcrop (Figs. 9 and 10). The dry soredial-like parts of the thallus lodge in the small nicks in the surface. On taking up water, they swell and exert a slight pressure on the shale around. Growth takes place, and, on further saturation, more pressure is exerted, and so on until the overhanging ledge is loosened. The hyphae penetrate still farther below the plate of shale, and by growth and swelling break the plate free from the substratum. The hyphae grow beneath the detached fragment, which eventually becomes raised from the substratum and incorporated into the lichen tissues. Hyphae then attack the substratum below in a similar manner, more plates are lifted from the surface, and these become incorporated into the thallus tissue by this simple mechanical action. As stated above, it does not appear from Miss Mellor's description that the glass flakes, incorporated in the glass-lichen tissues, were acted on chemically by the carbon dioxide of the lichens, but in the case of the included shale there is a very obvious chemical decomposition of the flakes, the latter changing in the tissues from blue-grey rock to a friable, brownish material. Although shale is only altered chemically by carbon dioxide when the latter is held in contact with the rock for a considerable time, the action must be considerably quicker on shale than on glass.

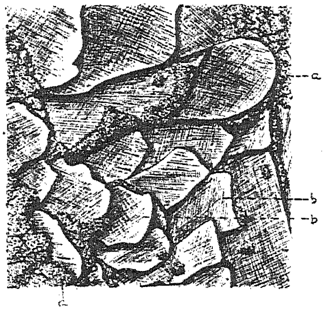


FIG. 9. *Lecidea lucida*. Surface view of colony. *a*, thallus; *b*, plates of shale pushed out of position.

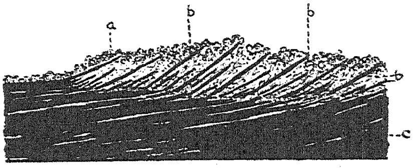


FIG. 10. *Lecidea lucida*. Longitudinal section of colony on shale. *a*, *L. lucida*; *b*, plates of shale pushed out of position, or partly detached; *c*, shale.

Miss Mellor states that the small pits on the glass surface were caused by the same simple mechanical action of the hyphae, separating the scales of glass on the surface 'previously chemically altered'. But the present writer attributes the formation of these pits to the initial mechanical action on the unaltered substratum by the haptera of foliaceous lichens, or to the action of crustaceous species. Leaving the latter for a future discussion, one can explain these pits in the glass as the result of the constant chipping away of the small areas immediately below the hapteral discs, as explained in the case of *Xanthoria parietina*.

Miss Mellor figures small thalli, e.g. *Lecanora murorum*, as occupying small depressions. It appears as if that species were responsible for the formation of the hollows, but this need not necessarily be the case. It may be that these pits in the surface were formed, as previously described, by pioneer lichens, and that *L. murorum* spores subsequently developed in the hollows, since these would offer favourable conditions for lichen development. One frequently found the spores of epilithic limestone lichens, and particularly *L. murorum*, developing in the old apothecial pits of endolithic species.

One has found in the early stages of development of the above species that there is a zone of gelatinous substance at the growing margin, in which the young hyphae ramify. This is very similar to the appearance one finds at the periphery of many species of crustaceous lichens. Miss Mellor states that in the earlier stages of development of lichen thalli there is less action on the surface than later on. Although this may be true for the polished surface of the glass, yet it may be that in the small depressions the young forms of *L. murorum* have their action accelerated by the presence of the gelatinous film, and also by the fact that the hollows offer a better foothold for the lichen. Water held by capillarity in these positions would be an important factor in development, for in the presence of moisture lichen thalli develop more rapidly. Consequently, the presence of small pits in the surface would lead ultimately to the more rapid recolonization of the glass. In this connexion it is interesting to note that Miss Mellor found that the glass next the lead casing of the windows was often the first to become corroded. Whether the lead casing fitted well down on to the glass or not, it always acted as a reservoir for water, and consequently lichens grew on the glass in the immediate neighbourhood of the lead. As a natural result of such development, the glass became corroded in these parts.

A point not dealt with by Miss Mellor is the possibility, in these regions, of a chemical reaction between the glass and the lead hydroxide, formed by the solution of lead in rain-water. Gaudin (3) states that lead corrodes more quickly than the glass, and may disappear in about fifty years.

In this discussion, although the chemical action of lichens on glass has been little considered, one does not exclude the possibility of such action on substrata. For example, in the case of endolithic limestone lichens (6, 9, 10), so far as one can judge at present, there seems to be strong reason for assuming a chemical reaction between the lichens and the limestone. Apparently the apothecial pits, hollowed out in the rock, are the result of the action on the limestone of carbon dioxide dissolved in water; yet the bark of trees is often hollowed out for the fruiting organs of corticolous crustaceous forms, and there seem to be very few, if any, plant substances which can act chemically on suberin. It has been noticed that in the regions of special growth, such as developing apothecia, there is unusual activity. This may be due to mechanical, as well as chemical action.

Miss Mellor states that, on detaching the apothecia of *Squamaria saxicola* from slate, there were little scales of that rock attached to the lower part of each apothecium, and on the surface of the substratum there were little holes where the apothecia had been. As a matter of fact, the present writer has found that the whole lower surface of certain crustaceous forms on shale was more or less caked with flakes of rock when portions were detached, besides the incorporation of shale flakes in the thallus. In the regions of the fruiting organs, however, the result was more evident.

By carrying farther the investigation on crustaceous thalli growing on rocks and trees, one hopes to obtain more knowledge of the relationship between lichens and their substrata.

Before closing this discussion, one would like to put forward the suggestion that this mechanical force, displayed by gelatinous or mucilaginous substances when contracting or expanding, might serve to explain other phenomena, such as the action of appressoria, sheaths of germ-tubes, or mucilaginous coats of certain fungal spores. Such repeated swelling and contraction may be due to other causes than alterations of temperature; for example, alterations in the acidity or alkalinity of the protoplasm or solutions with which the gelatinous substance is in contact, and these may cause lines of weakness to be set up in the impermeable cuticle of the host plant. It may be that through some such planes of weakness substances from the underlying cell exude and cause the vigorous growth of the germ-tubes which penetrate the cuticle at those points (8, 11). It may be that in the case of specific hosts there is some physiological relation between the mucilage of the fungus and the cuticle of the host. This relationship being present, the cuticle would be easily fractured and the plant readily infected.

SUMMARY.

1. Glass is corroded or chipped by gelatin drying on its surface.
2. Glass plates are perforated by the repeated application and drying of fresh gelatin films.

3. Superficial layers of shale are peeled off by the action of drying gelatin.

4. Gelatin masses in the form of foliaceous lichen thalli, attached to a shale substratum, seem to have a quicker action on the rock than films of gelatin in the form of crustaceous lichen thalli.

5. Thalli of lithophytic lichens are generally more or less gelatinous in the cortical region and attachment organs.

6. The mechanical action of lithophytic lichens on glass and shale can be explained by the powerful forces exerted by this gelatinous substance when contracting or expanding. Experiments with the foliaceous lichen, *Xanthoria parietina*, support this statement.

7. It was formerly held that the surface of the substratum first was altered chemically by the action of lichens, and then the parts of the decomposed material were separated by the mechanical action of the growing hyphae. It has been shown that these processes can be reversed, the initial stage being the mechanical disintegration of the unaltered rock by the lichen thalli, followed by the decomposition of the separated fragments.

The writer wishes to thank Professor J. Lloyd-Williams, D.Sc., and Miss M. M. Wells, B.Sc., for their kind help and encouragement during this investigation.

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Studies on Somatic Chromosomes.

I. Pairing and Segmentation in Galtonia.

BY

W. C. F. NEWTON,

Student of the John Innes Horticultural Institution.

With Plate IX and three Figures in the Text.

THE present study was undertaken to determine the nature and extent of the paired association of somatic chromosomes, which may be observed in the nuclear divisions of *Galtonia candicans*, Dcne., and of the closely related *G. princeps*, Dcne. A considerable importance is attached to the question owing to the difference of view which exists as to the correct interpretation of the double structures which are observable in telophase and interphase. One school of investigators regards these appearances as due to the premature splitting of the chromosomes, while another looks on the doubleness, so far as it is not due to faulty observation, as being occasioned by the pairing of paternal and maternal chromosomes. The actual existence of pairing, as anything more than a random association of chromosomes of similar size, has been questioned by Bonnet (1) and Nawaschin (9). The latter author based his conclusions on a study of *G. candicans*, the chromosomes of which are so sharply characterized as to allow of the recognition of individual pairs with certainty. The present investigation has led to conclusions different from those of Nawaschin, both as to pairing and as to the nature and importance of the structures called by him trabant chromosomes, to which considerable attention has been directed in recent years by him and by his pupils.

The material used consisted of root-tips and flower-buds of *G. candicans* and of root-tips of *G. princeps*. Fixations were made in a variety of fixing fluids, of which Flemming's stronger fluid and the modification of it recommended by Benda gave the best results. Merkel's fluid gave what was apparently an excellent fixation without any contraction of the cytoplasm, but the staining properties of the material were inferior. Benda's fixative

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gave a fuller fixation of the cytoplasm than either Flemming's or Merkel's, while the metaphase chromosomes are caused to appear more slender and longer. Examination of living material indicates that this result is the more nearly correct, but *Galtonia* is not a sufficiently favourable object for the study of living material to allow of a comparison being extended to all stages. The differences between the material from the three fixatives used were, however, only differences of degree, and the structures and stages described in this paper have been found with greater or less distinctness in material from each of them.

Sections were cut at thicknesses varying from 4 to 14 μ, and were stained in Breinl's triple stain, Flemming's triple stain, or Heidenhain's iron-haematoxylin. The first of these stains gave the best results.

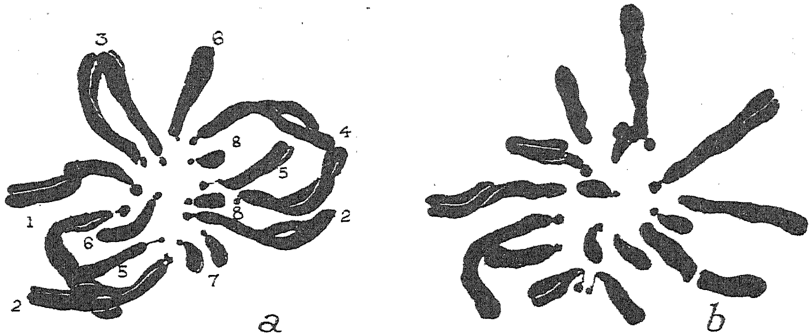
OBSERVATIONS.

The descriptions given, except where distinction is expressly made, apply to both plants. The differences are slight, but they are sufficient to give a characteristic appearance to the nuclei of the respective plants. This is especially the case in prophase, the successive stages of which appear much more obvious in *G. princeps*, the nuclei of which are appreciably larger.

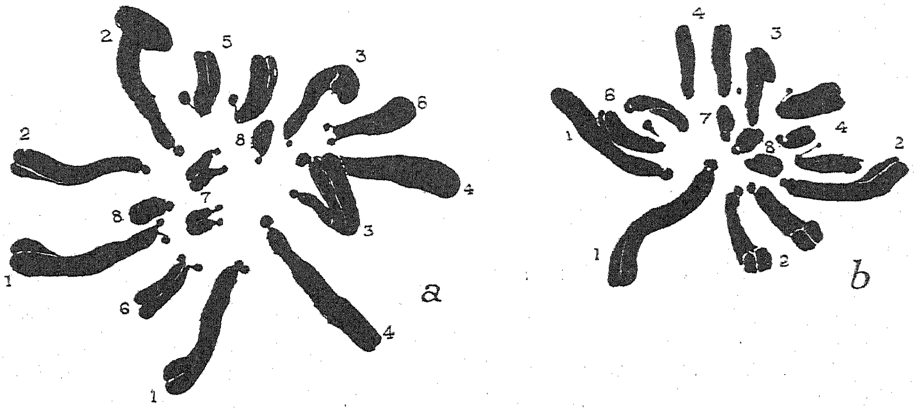
Metaphase. The relations of the sixteen slightly curved chromosomes can be seen in Text-figs. 1 and 2. The pairing of similar elements is by no means complete, and it is a rare exception to find a metaphase plate in which separation of the members of one or more pairs has not occurred. Indeed, if attention were confined to the metaphase, it would be impossible to maintain that the occurrence together of two similar chromosomes was anything more than a casual phenomenon. The separation that is involved is, however, only temporary. It takes place when the chromosomes, arranged roughly at right angles to the future equatorial plane, rotate into that plane at the beginning of metaphase. The pairs that are more centrally placed at the beginning of this process are especially liable to separation, so that one at least of the small pairs is usually affected. Any particular pair may or may not be affected.

The chromosomes fall into three well-marked groups in respect of length: the first group contains four pairs of long chromosomes; the second contains two pairs, the members of which are scarcely half as long; the third group contains two pairs of very short chromosomes. In number and relative length the chromosomes of *G. princeps* are identical with those of *G. candicans*. There is no difference in width between the chromosomes of the various groups in either plant.

Each chromosome, in all the plants examined, exhibits a subterminal constriction, dividing it into a relatively long distal and a very short proximal segment, the proximal end being that towards the spindle at metaphase. This constriction, which is in fact a very fine non-chromatic thread, is always situate at that point in the chromosome towards which the attachment fibre from the spindle is directed. In different chromosomes the proximal segments differ slightly in size, but there is no correlation between the size of the segment and that of the chromosome to which it belongs. The degree of separation of the segments varies between somewhat wide limits according to the stage of division. It is greatest in early anaphase. It also varies from pair to pair, and in the case of one of the pairs of intermediate length in *G. candicans* an extreme is reached in which the length of the non-chromatic thread approaches that of the distal segment. In *G. princeps* the corresponding pair of intermediate length also shows this condition, in which the proximal segment becomes what Nawaschin has called a trabant or satellite chromosome. There is not in



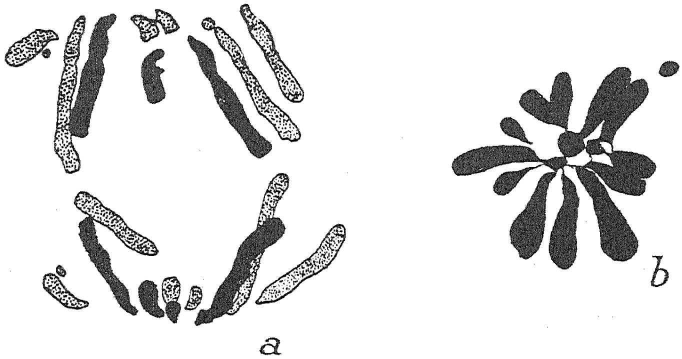
TEXT-FIG. 1. Chromosome complement of *G. candicans*. Chromosome pairs are numbered. × 2,700.



TEXT-FIG. 2. Chromosome complement of *G. princeps*. Pair 4 in *b* is much foreshortened. × 2,700.

this plant such a clear-cut distinction between the trabant and the normal condition of the segments as is found in *G. candicans.* Two other pairs at metaphase and one of the smallest pairs at anaphase have the segments of their members widely separated. It is evident, therefore, that a very close relation exists between the trabant condition and the simple segmentation of which it must be considered a development.

Anaphase and telophase. As the daughter chromosomes begin to separate at the position of the constrictions, a slight bending over of the proximal ends is produced, which persists throughout anaphase. The chromosomes become longer and more slender, an alteration that is accentuated in Benda material. In some cases a beaded structure is visible already in the metaphase chromosomes; in all cases the anaphase chromosomes show a moniliform structure. This appearance is not a prelude to the ragmentation of the chromosomes, as all traces of longitudinal differences in structure disappear at the beginning of the telophasic contraction stage which follows.



TEXT-FIG. 3. Early anaphase (*a*) and late anaphase (*b*) in G. *candicans.* In *a* the small chromosomes have reached the pole and are paired. In *b* the process of reassociation is almost complete. × 2,700.

It is at this stage that the pairing of homologous chromosomes, temporarily interrupted at metaphase, is re-established. As the polar area is reached the separated members of pairs approach one another, either internally or externally to the chromosomes which intervened when all were spread out in a single plane (cf. Text-fig. 3, *b*). The smallest chromosomes reach the polar area first, and, though usually separated at metaphase, they are now intimately paired. They take up a central position, and, as they can be recognized up to a late stage in telophase (cf. Pl. IX, Fig. 2), form convenient objects in which to follow the telophasic changes. The chromosomes become less precise in outline and appear in cross-section as central axes surrounded by a diffusely staining sheath. Fine processes run out from the axes to the limiting membrane of the sheath, as can best be seen in the longer chromosomes, certain of which project from the rest, giving to the newly-formed nucleus a crescentic outline. The surface limits of the individual chromosomes are soon lost in the formation of the nuclear vacuole, but the chromosome axes remain distinct, and in most cases undergo a median vacuolization which reduces them to the appearance of irregularly vacuolated double threads. Meanwhile there has been a gradual moving apart of the chromosomes, corresponding to a gradual increase in volume of the nuclear vacuole. It is not possible to recognize the occurrence of a median vacuolization in the case of every chromosome.

Interphase. The chromatic threads become finer as the nuclear volume increases, and the limits of the chromosome areas can no longer be distinguished with certainty, owing to the very slight differences in thickness between the axial threads and the anastomoses. The chromatic material is very evenly distributed, and there is no indication of chromocentres or prochromosomes.

Prophase. The prophasic changes are best followed in polar view. By the breaking down of the anastomoses somewhat irregularly vacuolated chromatic bands become distinguishable. These condense into polarized wavy bands with a median vacuolization, which gives them the appearance of being double structures. The final stage produces from these bands very slender spirally coiled threads, which show no trace of doubleness at their first formation. These spiral threads begin at once to straighten out, and as they do so their doubleness becomes apparent. Approximately the diploid number of threads which follow parallel courses in pairs can be recognized. This association is of a different order to the intimate association of the daughter halves which can be seen at the same time.

The so-called vacuolization which is described by some authors as effecting the separation of the daughter threads is really a result of that separation, and its appearance can be produced at any time up to metaphase by varying the fixation or the differentiation. It is a phenomenon of quite a different kind to the vacuolization of telophase. Like the anaphasic chromosomes, the daughter threads exhibit a moniliform structure which is correlated with the 'vacuolate' appearance referred to above. The most prominent features in this vacuolization are produced by the constrictions which are already visible. Inequalities in the distribution of the granules producing larger or smaller vacuoles occur also at other points in the chromosomes, as is shown in Pl. IX, Fig. 8. In the case of the constrictions it is certain that the vacuolization corresponds to definite features of the chromosome structure, constant in position and occurrence. It seems probable that this is also true of the remaining vacuoles, though it is not possible to control their occurrence in different nuclei with the same accuracy.

DISCUSSION.

The literature of chromosome pairing in both animals and plants is considered at some length in the paper of Metz (6), to which reference should be made. Apart from metaphase, the evidence so far as plants are concerned rests on the rather unsatisfactory basis of a numerical consideration of the prochromosomes. The closeness of the association described or inferred ranges from a tendency to pairing in *Crepis* (Rosenberg, 13), to complete fusion of the prochromosomes in pairs, reducing them to the haploid number, in *Musa sapientum*, var. *Dole* (Tischler, 16). The more certain method of following the fate of individual chromosomes, which is possible in *Galtonia*, shows that the association is a very loose one, except for a short period at anaphase, and that it is not of such a nature as to serve as an explanation of the double structures observed during telophase and interphase. During metaphase there is very frequent interruption of pairing, and it is obvious from the distribution of the chromosomes with satellite segments that it by no means follows that equal chromosomes in juxtaposition are necessarily members of the same pair. Thus in Cl. Müller's (8) figures of *Naias major* the chromosomes to which his pair 7 are attached are attributed to different pairs owing to the fact they are not associated. There is little doubt, however, that the so-called pair 7 consist of the satellite segments of a single pair of chromosomes (Tschernoyarow, 17). Similarly in Text-fig. 1, *a*, the size relations suggest that the chromosomes of intermediate length are paired, which the position of the satellite segments shows to be not actually the case. Such considerations led Nawaschin (10) to deny all significance to the occurrence of apparent pairing. Interruption of pairing at metaphase, however, also occurs in the Diptera, but much less frequently, as would be expected from the much more intimate nature of the association at all stages in these insects. The behaviour of the chromosomes in *Galtonia* suggests that the repulsion from the poles is much stronger than the attraction of the homologous chromosomes for one another, so that the pairing relations are broken if the two influences come into opposition. This opposition is obviously less likely to occur if the pairing in prophase, as in the Diptera, is very close.

The telophasic vacuolization is sufficiently regular in *Galtonia* to afford support to the theory that it involves the actual longitudinal splitting of the chromosomes. A definite proof, however, requires that it should be possible to demonstrate continuity between the telophasic vacuolization and the definitive split of prophase, and this has been found impossible in these plants.

The occurrence of constrictions at definite positions in the chromosomes of *Vicia Faba* was described by Fraser and Snell (4). Since that time considerable attention has been paid to their occurrence, notably by Nawaschin and his pupils (2, 9, 10, 17), and by Sakamura (14, 15). In the cases of *Vicia* and *Muscari* (Delaunay (2)) constrictions occur which are not situate at the point of attachment of the chromosomes to the spindle, but the large majority of the constrictions even in these cases resemble those of *Galtonia* in agreeing in position with the point of attachment. The relation of the constrictions to the processes involved in the production of the spindle is evidently close, but their appearance in prophase before that of the spindle shows that they are definite features of the chromosomes as distinct from mechanical results of stresses during division. It is, then, the structure of the chromosome which determines its relation to the spindle.

In the genus *Vicia*, Sakamura (14) found that the position of the constrictions afforded a means of distinguishing cytologically the different species. A similar systematic significance has been observed by the present author to be connected with the position of the constrictions in the genus *Tulipa.*

In *Galtonia* there is no reason to suppose that segmentation and the development of trabant segments is in any way connected with a gradual degeneration of the chromosomes, such as Delaunay (2) suggests has been the case in *Muscari.* It is rather to be considered that constrictions and trabants are expressions of a difference in degree of attraction between neighbouring structural units of the chromosomes which may well have significance as steps in a process of fragmentation of the chromosomes concerned. In this way might be explained the occurrence of aneuploid chromosome numbers—numbers, that is to say, which are not simple multiples of a basic number common to the circle of affinity to which the plant belongs. Thus the basic number in the case of the Liliaceae is three, and De Mol has produced evidence which tends to show that the numbers four and eight found in the genera *Hyacinthus* and *Bellevalia* have been produced by the transverse division of certain chromosomes of an original complement of three or six chromosomes of equal length. Numerically his argument would apply to *Galtonia*, but in this case the original chromosomes could not have been equal. Unequal chromosomes are, however, found in members of the Liliaceae with chromosome numbers still an exact multiple of three, and the fourteen chromosomes found in species of *Aloe* and *Gasteria* cannot be derived from twelve equal chromosomes, since eight are long, four are short, and two are of intermediate length.

Nawaschin (9) considered that the trabant segments were comparable to the sex chromosomes of insects, and he regarded the cases in which he observed a trabant in connexion with only one of the members of a pair as indicating sexual dimorphism in a hermaphrodite plant. The unequal pairs found by him are, however, rather comparable to the unequal pairs described by Wenrich in *Phrynotettix*, which also differ owing to the absence of a terminal segment in one member of the pair.

SUMMARY.

1. The nuclei of *Galtonia candicans*, Dcne., and of *G. princeps*, Dcne., exhibit eight pairs of chromosomes, divisible into three groups, consisting respectively of four pairs of long, two pairs of very short chromosomes, and two pairs of chromosomes of intermediate length.

2. The chromosomes in each plant exhibit a subterminal constriction, consisting of a very fine non-chromatic thread, which divides the chromosome into a short proximal and a comparatively long distal segment. The constrictions are constant in position at the point of attachment of the chromosomes to the spindle fibres. The constrictions are visible in early prophase as more sharply marked examples of the chromomere structure of the chromosome.

3. In the case of one pair of chromosomes in *G. candicans* and of three pairs in *G. princeps* there is a considerable degree of separation between the segments. In such cases certain authors have distinguished the smaller segments as satellite or trabant chromosomes. There is a more sharply marked distinction between the trabant and the normal condition of the segments in *G. candicans* than in *G. princeps*. In neither plant is there any reason to suppose that the trabants are in process of disappearance.

4. Pairing of the chromosomes is found to be much interrupted at metaphase when chromosomes of similar size lying side by side are not always members of the same pair as distinguished by the segmentation. True pairing is found in anaphase when reassociation of chromosomes separated at metaphase takes place. Pairing persists as an approximate parallelism throughout telophase and prophase. It is easily distinguishable from the doubleness produced by the longitudinal split owing to the greater intimacy of the association of the threads produced in the latter case.

5. An apparent doubleness of the chromosomes in telophase and early prophase, independent of the paired association, was observed, but it was found impossible to demonstrate the continuity of this doubleness with that occurring in slightly later prophase.

This investigation was begun in the laboratory of Birkbeck College during my tenure of a grant from the Department of Industrial and

Scientific Research. It was completed at the John Innes Horticultural Institution.

My thanks are due to Professor Dame Helen Gwynne-Vaughan and to Mr. Bateson for unfailing interest and assistance.

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DESCRIPTION OF FIGURES IN PLATE IX.

Illustrating Mr. W. C. F. Newton's paper on Somatic Chromosomes.

All figures are of *Galtonia candicans*, except Fig. 11, which is of *G. princeps*. They were drawn with the Abbe camera lucida using a Leitz.

Fig. 1. Chromosomes in anaphase showing beaded structure.

Fig. 2. Telophase. All the chromosomes distinct. Vacuolization median.

Fig. 3. Telophase. Progressive vacuolization has obscured the limits of some of the chromosomes.

Fig. 4. Late telophase.

Fig. 5. Prophase. Irregular double bands mark the position of the reappearing chromosomes.

Fig. 6. Prophase. Spirally coiled and polarized chromosomes.

Fig. 7. The same stage as Fig. 6, but further differentiated.

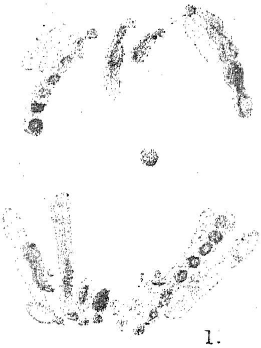
Fig. 8. Prophase. Paired granules in the daughter chromosomes. Position of the constrictions indicated by slender threads.

Fig. 9. Prophase. Constrictions and terminal granules.

Fig. 10. Prophase. Paired and longitudinally split chromosomes.

Fig. 11. Prophase. Pairing, anastomoses, constrictions, and trabant segments.

Fig. 12. Polarization of chromosomes.



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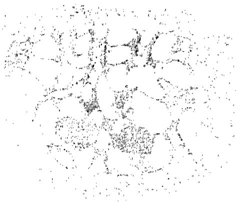

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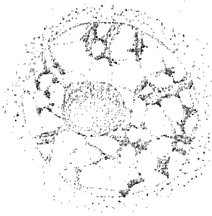

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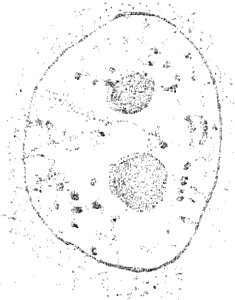

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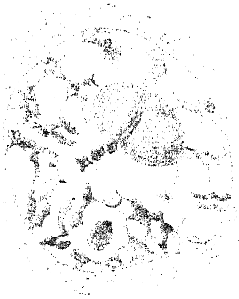

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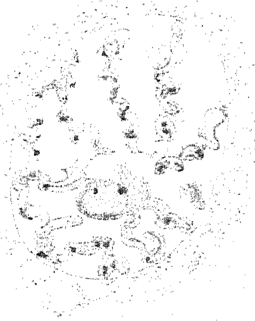

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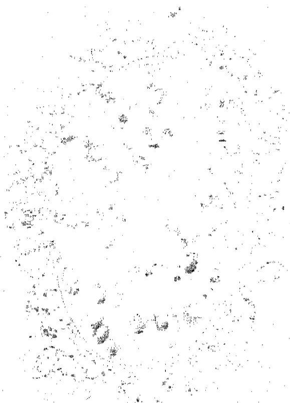

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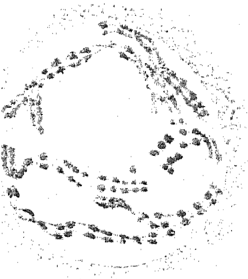

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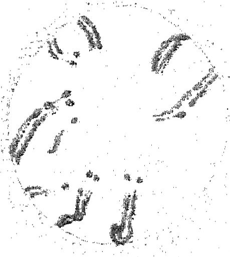

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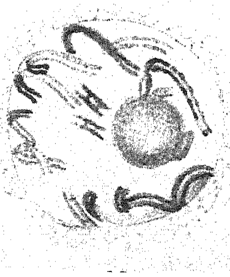

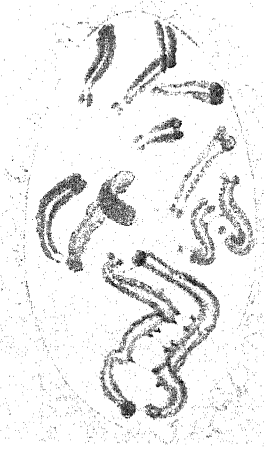

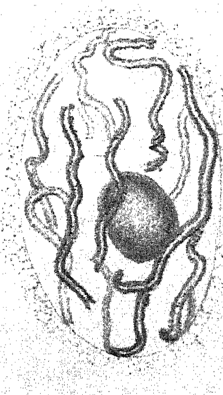

12.

NOTES.

AN ABNORMAL PRO-EMBRYONIC BRANCH OF CHARA VULGARIS, L.—In the course of work on the Characeae, which is in progress, practically all the deviations from the normal, already described in the literature, have been seen either on plants growing in their natural habitat or on pieces under experiment. A pro-embryonic branch of *Chara vulgaris*, L. (*C. foetida*, Braun),[1] showing a tendency to continue apical growth as a shoot, is of some morphological importance, and since no record has been found of the occurrence of such a branch a short account of it is given here.

A normal pro-embryonic branch[2] is like the pro-embryo[3] of the sporeling, both in its development and mature construction. In both, a filament of cells with limited apical growth is first formed; this is recognizable later as the pro-embryo tip (Vorkeimspitze). By divisions in one of the lower cells, two nodes separated by an internode are formed. Whereas rhizoids originate from the lower node, the first shoot of unlimited apical growth starts as a lateral bud at the upper node, along with some 'leaves'. The chief interest of the branch to be described is that the pro-embryo tip was not a cell-row of limited growth, but had undergone divisions like the apical cell of a shoot.

This abnormal branch was found on a node, being used in a series of experiments, in which the bases of the axillary branch and of its subtending 'leaf' were cut out from single nodes of *Chara vulgaris*. These nodes were then put in test-tubes which were filled with tap-water, plugged loosely with cotton-wool, and kept in a greenhouse. Under these conditions pro-embryonic branches soon developed from the injured nodes. This account will be limited to the node which gave rise to the abnormal branch. Within sixteen days from the beginning of the experiment the first pro-embryonic branch (Fig. 1, *pr.*^{1}) had appeared, and in two months' time there were altogether four, in various stages of development (Fig. 1, *pr.*$^{1-4}$). All had the stem-node (Sprossknoten) differentiated, but none had a root-node (Wurzelknoten); this is a very common deviation from the normal structure in pro-embryos arising as adventitious branches. In these cases there was only a single cell below the stem-node, but others have been seen with two or even three. In addition to these four branches, however, there was a more abnormal branch (Fig. 1, *br.*, and Fig. 2). It had originated from one of an irregular group of cells on the upper side of the base of one of the 'leaves' bordering on the wound (Fig. 1, *sc.*). The basal, green cell was

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[1] I am indebted to J. Groves, Esq., F.L.S., for naming the plant.

[2] Pringsheim, N.: Über die Vorkeime der Charen. Monatsber. d. K. Akad. d. Wiss. zu. Berlin, math. phys. Kl., 1862. Über die Vorkeime u. die nacktfüssigen Zweige der Charen. Jahrb f. wiss. Bot., 1862.

[3] Bary, A. de: Die Keimungsgeschichte der Charen. Bot. Ztg., 1875, xxxiii. 377.

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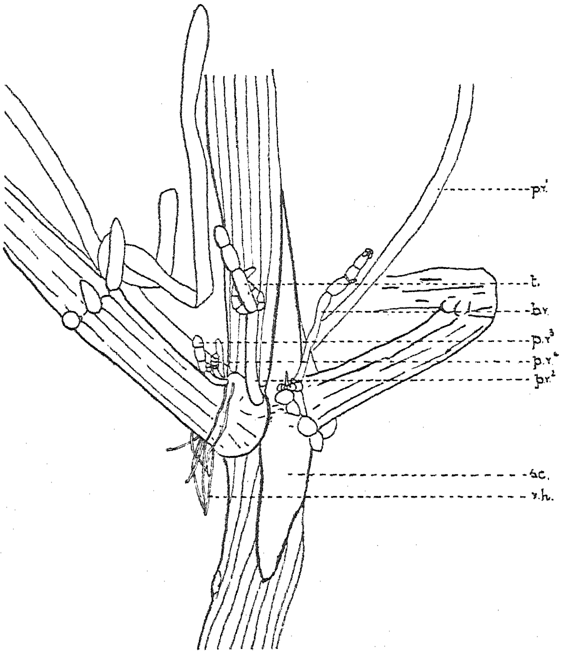
separated from the lowest node (Fig. 2, *s.n.*) by another shorter cell. This node, which apparently corresponded to the stem-node of a pro-embryonic branch, had, however, no sign of a lateral branch. Only two of its segments had developed into 'leaves', and these were both small and rudimentary (Fig. 2, *l.*). The pro-embryo tip of *Chara vulgaris* usually consists of a row of three cells, but in this case, instead of the third and uppermost cell, there was a node (Fig. 2, *n.*), and above this a dome-shaped apical cell, exactly like the apical cell of a shoot (Fig. 2, *v.*). From the node (Fig. 2, *n.*) two 'leaves' (Fig. 2, *ll.*) had grown. They were small and rudimentary, each being made up of only two cells. As at the other node, there was no sign of a lateral branch.



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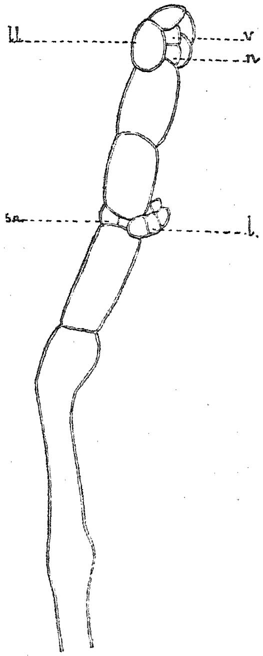

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FIG. 1. *Chara vulgaris.* Isolated node with developing pro-embryonic branches. × 10. *br.*, abnormal pro-embryonic branch; *pr.*¹⁻⁴, pro-embryonic branches; *rh.*, rhizoids; *sc.*, wound *t.*, pro-embryo tip.

FIG. 2. *Chara vulgaris.* Abnormal pro-embryonic branch. × 75. *l.*, leaves of stem node *ll.*, leaves of apical node; *n.*, apical node; *s.n.*, stem node of pro-embryonic branch *v.*, apica cell.

This branch evidently corresponds to a pro-embryonic branch and therefore to a pro-embryo, while by the transformation of the pro-embryo tip it approaches the construction of a shoot with its apical growth. The only case at all comparable with this appears to be one described by Nordstedt.[1] In his specimen, as a result of the suppression or destruction of the pro-embryo, one of the other cells of the first node, at the apex of the oospore, had grown out into a branch. This branch resembled a shoot with apical growth, but no appendages had been formed at the lowest node. A comparison of Nordstedt's specimen with the one described here would seem to indicate that both were structures intermediate between a pro-embryo and a shoot with apical growth, but while Nordstedt's branch had characters more like those of a shoot, this abnormal branch, judging from its place of origin and the general arrangement of its cells, can be regarded as derived by the transformation of a pro-embryo tip.

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[1] Nordstedt, O.: Några iaktagelser öfver Characeernas gröning. Lunds Univers. Årsskrifter, ii, 1865.

This is the only example which has been met with so far, and it is impossible to make any suggestion as to the factors which caused its development.

KATHLEEN M. DREW.

CRYPTOGAMIC RESEARCH LABORATORY,
UNIVERSITY OF MANCHESTER.

A NOTE ON THE DORMANCY OF THE SEEDS OF LATHYRUS MARITIMUS.—*Lathyrus maritimus*, Big., the sea pea, is a perennial occurring locally on the coast of Britain, principally on pebbly beaches. The material used for the investigation here recorded was obtained by one of us from the Chesil Bank, Dorset, in the neighbourhood of Abbotsbury, during the excursion to the Bank of the British Ecological Society on August 26, 1922. We are indebted to Professor F. W. Oliver for pointing out some of the characters of this interesting plant, including the character which forms the subject of the present investigation—the dormancy of the seeds.

The seeds of *Lathyrus maritimus*, when mature in the pod, are almost spherical, about 0·22 cm. in diameter, olive green to brown in colour, with an average weight of about 0·039 grm. On an average about six seeds are contained in a pod. The specific gravity of the seeds when shed is less than unity, so that the seeds float on distilled water, and consequently on the heavier sea-water.

The seeds, when sown under conditions favourable to germination, show no tendency to germinate. This dormancy may be due to a variety of causes,[1] but, as with many Leguminosae, the seeds possess a tough coat, and it is a likely possibility, as with many other Leguminosae, that the dormancy is due to the impermeability of the seed-coat to water.

Experiments were therefore made in which the seeds were placed in a vessel containing distilled water and were weighed at intervals. These experiments revealed the fact that the seed-coat is exceedingly impermeable to water. For example, twenty seeds were placed in distilled water maintained at 25° C. and were weighed at intervals. The water was occasionally renewed, and after two months in a thermostat the bottle containing the seeds was removed to a table in the laboratory, where it remained during the further course of the experiment at laboratory temperature (about 16° C.). After the lapse of twelve months the seeds had not absorbed any water whatever. The weights of the seeds actually found in one experiment are given in Table I. Any difference in weight at different times is clearly within the range of experimental error arising from slight differences in the degree of drying of the seeds.

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[1] Cf. Crocker, Amer. Journ. Bot., iii. 99, 1916.

TABLE I.

Weight of twenty seeds of Lathyrus maritimus after various times in contact with distilled water.

Time in days.	*Weight in grammes.*
0	0·801
1	0·802
5	0·801
14	0·8015
52	0·8015
87	0·800
124	0·800
162	0·8005
202	0·800
381	0·801

In order to make certain that the incapacity of the seed to absorb water is due to the impermeability of the seed-coat and not to some other unsuspected cause, the coats of a number of seeds were chipped in places so that the continuity of the coat was broken. Such seeds, when immersed in water, absorbed the latter at once with considerable rapidity. Two experiments may be quoted from a number giving similar results. The coats of twenty seeds were chipped in places away from the radicle and soaked in water. They were removed from the water at intervals, dried rapidly and thoroughly, weighed, and returned to the water. The results are shown in Table II. The time taken for the weighing is not included in calculating the time of immersion of the seeds. It will be observed that under these conditions absorption of water commences at once and continues for four or five days, by which time the seeds may have absorbed water to the extent of 230 per cent. of their original weight.

TABLE II.

Absorption of water by twenty seeds of Lathyrus maritimus with chipped seed-coats.

Time in hours.	*Weight in grammes.*		*Gain in weight in grammes.*	
	Sample 1. 23° C.	Sample 2. 25° C.	Sample 1.	Sample 2.
0·0	0·7840	0·7758	0·0	0·0
0·22	0·8229	—	0·0389	—
0·53	0·8829	—	0·0989	—
1·13	1·1179	—	0·3339	—
1·60	1·3750	—	0·5910	—
23·0	—	2·5105	—	1·7347
47·3	—	2·5455	—	1·7697
71·8	—	2·5478	—	1·7720
123·0	—	2·5654	—	1·7896

Seeds so treated when sown in soil in pans in the laboratory germinate without delay, and after the lapse of seven to fourteen days the epicotyls of the seedlings are visible above the surface of the soil. There thus can be no doubt that the dormancy of the seeds of *Lathyrus maritimus* is due solely to the resistance to the passage of water offered by the seed-coat.

It is of interest to discover, if possible, to what the impermeability to water of the seed-coat of *Lathyrus maritimus* is due. To this end samples of seeds were treated with different reagents for various times, and then the swelling capacity examined by placing the seeds in distilled water; in this way it may be determined whether the solvent removed any substance to the presence of which the impermeability of the seed-coat is due. Ether, alcohol, chloroform, and petrol-ether were employed, the seeds being immersed in them at laboratory temperature for various times from five minutes up to twenty-four hours, but in no case would the seeds absorb water after such treatment. That this result was not due to penetration of the solvent and subsequent killing of the cells was shown by chipping such treated seeds, when they were found to be capable of swelling in water and of subsequent germination. It thus seems unlikely that the impermeability is due to impregnation of the cell-walls of the seed-coat with a fatty substance.

On the other hand, soaking the seeds in concentrated sulphuric acid for thirty-five minutes rendered them capable of absorbing water and swelling. Ten seeds so treated were planted after swelling in water, and all germinated.

Treatment of seeds with boiling water for five minutes also rendered them capable of swelling in water, but this treatment kills the embryo, for seeds subjected to this process failed to germinate. In water at 90° C. three seeds out of ten were rendered capable of swelling after five minutes, one more after fifteen minutes, and the remaining six after twenty-five minutes' treatment, but in all cases the seeds were no longer viable. Longer treatment at 80° C. produces the same result. After immersion in water at 70° C. for one hour, two seeds out of ten swelled, and one more after three hours' immersion, but here also the seeds were killed by the treatment.

Immersion of the seeds in sea-water for short periods up to twenty-four hours is without any effect on the dormancy of the seeds, but Professor Oliver informs us that he has observed the germination of the seeds after prolonged treatment with sea-water lasting many months.

It thus seems likely that the impermeability of the seed-coat is due to the structure, possibly the colloidal structure, of the cellulose-pectin constituents of the cell-walls. These, as shown by microscopical examination, swell up in concentrated sulphuric acid and in hot water, and then become permeable, perhaps in much the same way as artificial membranes of collodion are made permeable if treated in their preparation with alcohol-water mixtures, whereas the air-dry membrane is impermeable to water.[1]

A microchemical examination of the cell-wall supports this view. The cell-wall strongly reacts with the usual cellulose reagents—iodine, chlor-zinc iodide, concentrated sulphuric acid and iodine, and concentrated sulphuric acid. On the other hand, it does not give a reaction for lignin, while treatment with alkanin, osmic acid, and scarlet R, while indicating the presence of a small amount of fat, suggests that this is present in only relatively small quantity.

It is worth noting that the swelling of the seeds of *Lathyrus maritimus* in water cannot be exclusively a simple osmotic phenomenon. As the dry seed will absorb water when the continuity of the seed-coat is broken, clearly the seed-coat cannot be acting as a semi-permeable membrane in this absorption. Also, as the seeds will absorb water after treatment with hot and even boiling water, which kills the cells, it is extremely improbable that the limiting layer of the protoplasm can be functioning as a semi-permeable membrane, as killing a cell always appears to involve the breaking down of the semi-permeable properties of the protoplasm. It would therefore appear that the absorption of water by the seeds of *Lathyrus maritimus* is due, at least in part, to swelling of the colloidal constituents of the cells of the embryo.

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[1] W. Brown, Biochem. Journ., ix. 591, 1915. If this view is correct, it is possible that seed freshly harvested and sown at once, before the seed-coats become too dry, may absorb water and germinate forthwith. Our material has not enabled us to examine this possibility.

From an ecological point of view it is interesting to speculate on the way in which the seeds of *Lathyrus maritimus* come to germinate under natural conditions, having regard to the impermeability of the intact seed-coat. It may, of course, be that reproduction chiefly takes place vegetatively by means of rhizomes, and that reproduction by seeds only takes place occasionally. Or possibly the seeds are rendered capable of absorbing water by long contact with sea-water. However, it seems by no means impossible that water-absorption and germination are rendered possible by mechanical damage to the seed-coats resulting from the knocking and rubbing against them of the pebbles which are present in such abundance, at any rate in some of their natural habitats. Although stated by Babington[1] to be a rare plant, *Lathyrus maritimus* is locally so abundant on parts of the Chesil Bank as to give a green colour to the Bank in places. The extreme roughness of the seas which break on the bank in autumn and winter is well known, and during gales and heavy seas the pebbles of the Bank are hurled about and against one another with very considerable force, and the coats of the seeds of *Lathyrus maritimus*, shed and lying among the pebbles of the Bank, may very conceivably receive sufficient damage to enable the easy entry of water, and so permit the subsequent germination of the seed. Such a suggestion is merely speculative, but it appears at least credible, especially in view of the fact that it is where these conditions of a pebble habitat and rough seas occur that the plant is present in abundance.

WALTER STILES.
MARGARET E. DELLOW.

UNIVERSITY COLLEGE, READING,
11 *June*, 1923.

A METHOD OF INOCULATING THE APPLE.—During the course of an investigation into the diseases incident to fruit in cold storage undertaken for the Food Investigation Board, Department of Scientific and Industrial Research, it became necessary to carry out a large number of inoculations of apples at yearly intervals, using fungi originally isolated from diseased apples, with a view to determining whether these fungi were able to attack the living tissue when the apples were kept at relatively low temperatures (1–5° C.), and whether certain varieties of apples were more susceptible to fungal attack than others.

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[1] Manual of British Botany, Ninth Edition, London, 1904.

In the earlier experiments a method somewhat similar to that devised by S. G. Paine for inoculating potatoes with bacteria was adopted (see Report of the International Phytopathological Conference, Wageningen, Holland, now in the press). A cylindrical cavity of the required depth is bored in the apple by means of a steel auger of the requisite gauge, previously dipped in absolute alcohol and flamed; the inoculant is then inserted. In the meantime a sterile cork-borer has been plunged deeply into the flesh of another apple and withdrawn again, and should contain after withdrawal a cylindrical core of tissue. The borer should be selected to cut out a plug that will just fit the hole made in the first apple. If the plug is too long for the hole, it is cut off flush with the skin. The disadvantages of this method are, firstly, the use of the flesh of a second apple which may differ chemically from the first, and secondly, since the plug is cut to make it level with the skin of the apple, the cut end of the plug is unprotected from loss of water through evaporation.

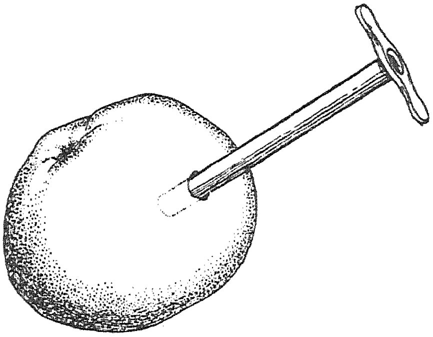
FIG. 1.

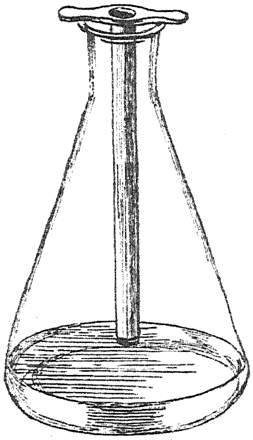
FIG. 2.

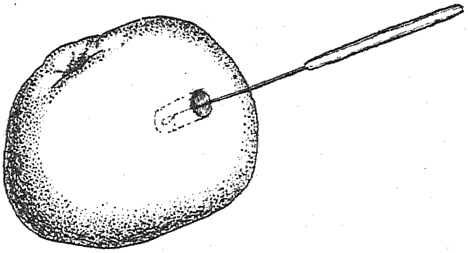
FIG. 3.

A modification of this method was ultimately adopted which obviates the use [illegible] an auger, and is not subject to the above-mentioned disadvantages. A cylindr plug was removed by a cork-borer, and after inoculation the same plug was rep in the cavity. When inoculations are undertaken on a large scale the operation is best performed by two persons. The first person plunges a sterile cork-borer into the apple to the required depth (Fig. 1); the borer is then rapidly removed, and with care in manipulation should contain a plug of apple tissue and leave a cylindrical cavity in the apple. Immediately after removal the borer is suspended over a 0·1 per cent. solution of mercuric chloride contained in a conical flask (Fig. 2). The apple is then

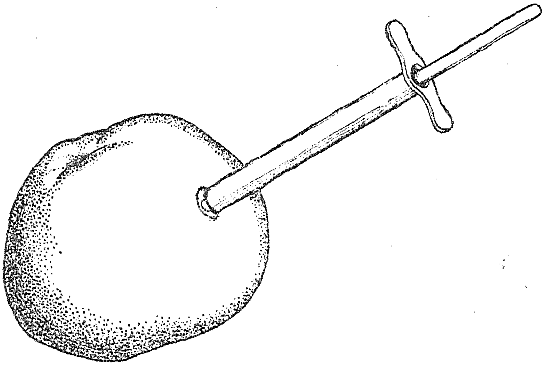

FIG. 4.

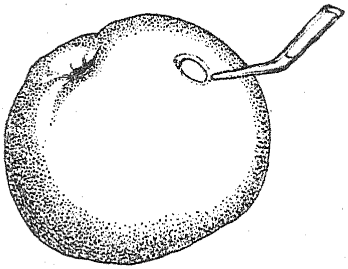

FIG. 5.

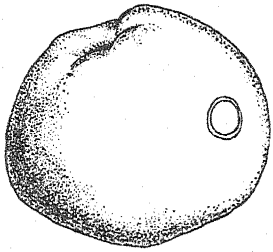

FIG. 6.

sed to the second person who inserts the inoculant (Fig. 3). After inoculation the le is returned to the first person, who applies the borer to the aperture in the apple pushes the plug home by means of a glass rod (Fig. 4). Melted paraffin is then led round the margin of the plug by means of a small glass dipper (Fig. 5). Fig. 6 shows the apple after sealing. If the apple is inoculated at more than one point the process is repeated. Finally the apple is passed to the second person, who lightly rubs its whole surface with clean cotton-wool previously moistened with absolute alcohol. is then wrapped in sterile grease-proof paper (sterilized in the hot-air oven at 180° C.) which is sealed with adhesive paper and labelled. It is possible to prepare twenty apples per hour, inoculated at two points each, by this method.

This method cannot be adopted for the inoculation of potatoes. The successful oval of a plug depends on two factors: (1) the friction between the plug and the —the greater the friction the more easily is the plug withdrawn: the apple plug possesses a slightly rough exterior and clings to the wall of the borer; the potato plug, on the other hand, is smooth and soapy and does not cling, the friction of the skin being insufficient to bring about its removal; (2) nature of the tissue: the tissue of the potato is tough and elastic, whereas that of the apple is more delicate and brittle. In the case of apples failure to remove plugs did not amount, after a little practice, to more than 3 per cent., whereas with potatoes it was impossible to remove an undamaged plug.

Uninoculated plugged control apples kept for six months at 1° C. still retained their waxen seals intact and healthy unshrunken plugs. In some cases where bitter pit had developed in control apples, small brown spots were found in the tissue of the plugs.

The method of sterilizing the apples by the application of absolute alcohol proved very successful. During the season 1922–3, less than 1 per cent. of the apples (1,500) inoculated became accidentally infected with *Penicillium*, *Botrytis*, and other moulds during the time they were stored in the 1° C. chamber at the Low Temperature Research Station, Cambridge.

Some evidence has been obtained that the actual lesion of the tissue induces an alteration in the physiological condition of the cells of the tissue adjacent to those of the plug. Thus the incidence of 'internal break-down' is arrested in the neighbourhood of the boring, especially in Bramley's Seedlings kept at low temperatures.

K. GRANGER.
A. S. HORNE.

IMPERIAL COLLEGE OF SCIENCE
AND TECHNOLOGY.

OSMUNDITES KIDSTONI, STOPES.—Some years ago Dr. Stopes[1] described and figured a fossil plant from Queensland, probably of Cretaceous age, which she named *Osmundites Kidstoni*. As it shows a solid stele, while the leaf-bases are of the same type as in other Osmundaceae, it is considered by this author as an intermediate form—a missing link—between the protostelic Osmundaceae and the more specialized members of this group.

Considering the figures, I felt some doubt whether the axis did or did not belong to the coating of leaf-bases. I am much indebted to Dr. Smith Woodward, F.R.S., Keeper of the Geological Department of the British Museum (Natural History), who kindly permitted me to study the specimens. The examination confirmed my opinion, which is based upon the following arguments. (1) The distribution of the petioles, shown in Pl. II (l. c.). If the small stem, which is visible in Figs. 4 and 5, really belongs to the same plant as the leaf-bases, it might be expected that those leaf-bases which are present in the same slide would be arranged round the stem. This, however, is not the case. The petioles are lying close together, forming a thick coating as in the other Osmundaceae. Only a part of this coating is present in the specimen; the planes of symmetry of the leaf-bases meet outside the slide where the axis might be expected. (2) The condition of preservation of the petioles. The leaf-bases lying near the stem, which is clearly marked off from the surrounding tissues, are crushed, as if the stem had penetrated into the mass of leaf-bases. (3) The structure of the stem. As the coating of leaf-bases is well developed, it is very probable that the stem belonging to them possessed short internodes, as in the other Osmundaceae, and numerous leaf-traces passing through the cortex and fusing with the central bundle might have been expected. Besides, connexion between the outer parts of the cortex and some leaf-bases might have been observed. This, however, is not the case. The stem is clearly marked off from the surrounding leaf-bases, and no leaf-traces passing through the cortex could be observed. The tissues of the cortex have wholly disappeared; only the central xylem is present, surrounded by a layer of large, strongly-marked cells. The structure of the six-rayed star of primary wood is the same in all the slides; no fusion with an incoming leaf-trace is visible. These characters point to the possession of long internodes, quite different from the structure usual in the Osmundaceae. Moreover, its size, when compared with that of the vascular bundles in the leaf-bases, is smaller than in the protostelic Osmundaceae, as *Thamnopteris* and *Zalesskya.*

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[1] Annals of Botany, vol. xxxv, 1921, pp. 55–61, Pl. II.

Summary. The specimen which has been described as *Osmundites Kidstoni* consists of a coating of leaf-bases of a species of *Osmundites* closely allied to that mentioned by Kidston and Gwynne-Vaughan,[1] and a piece of a stem with long internodes which penetrates into this packing of leaf-bases, without belonging to them; the affinity of the stem is uncertain.

O. POSTHUMUS.

BOTANICAL LABORATORY OF THE
STATE UNIVERSITY, GRONINGEN.

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[1] Transactions of the Royal Society of Edinburgh, vol. l, 1914, p. 478, Text-fig. 4.

The Anatomy of the Buds of Coniferae.

BY

FRANCIS J. LEWIS, D.Sc.,

AND

E. S. DOWDING, B.Sc.,

Botanical Laboratories, University of Alberta, Canada.

With twelve Figures in the Text.

DURING an examination of the food reserves in the bud of *Picea canadensis* in 1921, the structure appeared to be so interesting that it was decided to make a comparative study of several genera and species of conifers, native to Western Canada.

There appears to be but little literature available dealing with the structure of vegetative buds of conifers, most observers having confined their attention to the seedling, mature vegetative organs, and reproductive structures, and there is even difference of opinion as to the degree of differentiation of tissues at the growing point.

Many questions of great physiological interest attach to such structures. A number of the Coniferae reach farther north and attain higher altitudes than any other trees, and are exposed for many months in the year to temperatures ranging down to −60° C. The storage of food materials, water relationship, and the phenomenon of dormancy can only be successfully studied if the tissue arrangement of the buds is previously known.

All the trees described in this paper are members of the Abietineae, species of the following genera having been investigated: *Picea*, *Pseudotsuga*, *Larix*, *Abies*, *Pinus*.

Material was collected during the spring, summer, and autumn, except in the case of some species which only occur at high elevations in the mountains. After having been fixed and preserved in alcohol, microtome sections by the paraffin method were made. In spite of several hours' exhaustion under a rotary air-pump before passing on from the alcohol, much of the material proved to be very refractory, owing to the closely overlapping bud scales and the large amount of air contained in the tissues. In some cases this difficulty was got over by dissecting off the bud scales, but in other

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material the best method of obtaining complete sections of the delicate structures was by means of a Spencer carbon dioxide freezing microtome. This gave excellent results, particularly with fresh material, and sections could be cut nearly as thin as those obtained by the paraffin method.

Picea canadensis, (Mill.) B. S. P.

This is one of the most widely distributed trees in Canada, and reaches elevations of 6,000 to 7,000 feet in the mountains.

In Western Canada the bud begins to elongate in May, and the young shoot is usually fully formed by the middle of June.

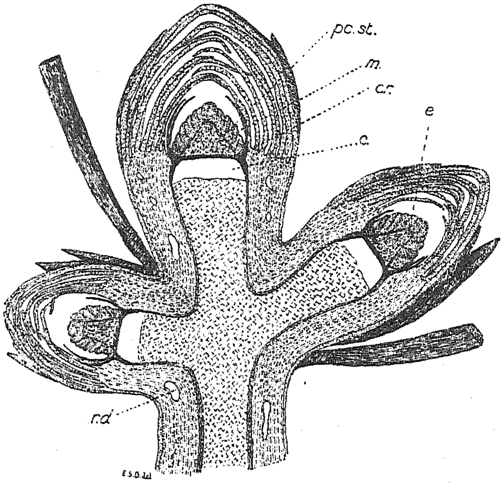

FIG. 1. *Picea canadensis.* Terminal and lateral buds in February. *pc. st.*, procambial strand; *m.*, medulla of next year's shoot; *cr.*, crown forming boundary between successive yearly shoots; *c.*, cavity formed by breaking down of medulla; *r.d.*, resin duct.

The general arrangement of the tissues of the mature winter's bud may first be described.

During the winter the bud is invested with brown chaffy scales forming a conical covering which rises on the inner side, about 3 mm. beyond the apical cluster of young leaves. The bases of the inner scales, which contain chlorophyll, are fused together into a tubular structure rising to a level of about half-way up the conical mass of young leaves. The general arrangement is shown in Fig. 1. The young leaves are borne on a cone of parenchyma (Fig. 1, *m.*), the future medulla of the next year's shoot. This is surrounded by a series of procambial strands (*pc. st.*) arranged in a circle and continuous with those entering the young leaves. The future medulla is bounded below by a deep layer of thick-walled cells which we have called the 'crown' (*cr.*). The old medulla below the crown breaks down during the autumn and forms a cavity (*c.*) of considerable size.

The following points may now be described in detail: (*a*) growing point, (*b*) medulla of the bud and procambial strands, (*c*) the crown.

(*a*) *Growing point.*

Different accounts have been given by several investigators as to the presence of the dermatogen, periblem, and pleurome in the growing point of the Gymnospermae. In the Abietineae, according to de Bary (1), the three layers run together into a common initial group which occupies the extreme apex; a separation into three layers first appears at some distance beneath this in Cycas, and more clearly in the Abietineae. Koch (2) found that the dermatogen is not constantly distinct, the apical layer undergoing periclinal divisions and thus contributing to the cortex as well as to the epidermis. Neither does this observer find any distinct initial group for the central cylinder, for the supposed pleurome gives rise to the pith only, which is the first tissue to be differentiated; the procambial strands arising from the so-called periblem.

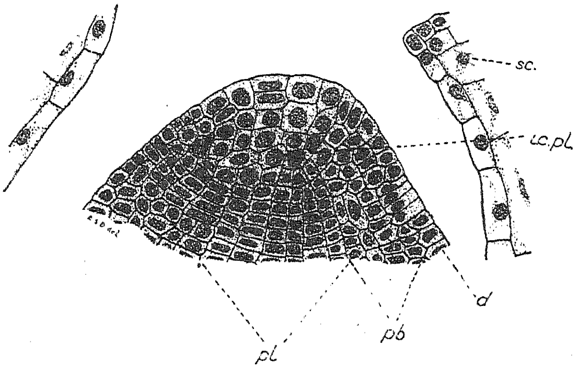

FIG. 2. Growing point, July. *sc.*, scale; *d.*, dermatogen; *pb.*, periblem; *pl.*, pleurome; *i. c. pl.*, initial cells of pleurome.

Our observations agree to some extent with those of Koch cited above, and lead us to the conclusion that there is no sharp differentiation between the dermatogen and the periblem, but that the pith is formed from a special group of cells some distance below the apex of the stem. This condition of things also applies to *Abies* and *Pseudotsuga.*

The apex of the stem in early July is shown in Fig. 2. At this time the shoot is fully elongated and the bud for the ensuing year is beginning to be formed. The dermatogen and periblem are not clearly separated, periclinal and anticlinal cell divisions taking place in the tissue from *d.* to *pb.* The pleurome is formed by the division of a group of cells at *i. c. pl.* (Fig. 2), and meristematic divisions occur for some distance down, and it is the lower layers of these cells which later thicken up in a characteristic way to form the crown. Meristematic activity spreads out from this level through the periblem to produce the lateral extensions of the crown, which is fully formed in August.

(*b*) *Medulla of the Bud and Procambial Strands.*

The medulla above the crown consists of a conical mass of regularly arranged parenchyma. Immediately above the crown the cells are flattened, but elsewhere they are iso-diametric, terminating above in a group of large initial cells shown in Fig. 2 (*i. c. pl.*). The first few rows of cells below the initial group are clearly meristematic. The whole of the medulla above the crown, except the initial cells, is greenish in colour in sections from living material and is sharply marked out from the colourless cells of the future cortex (Fig. 2, *pb.*). The mature medulla of the shoot is interrupted at intervals by transverse bands of sclerotic cells, but these cease some distance below the crown. Similarly, they do not appear until the bud has elongated during the early part of the summer, and are thus absent immediately above and below the crown. In the late summer or autumn the medulla breaks down to form a large cavity immediately below the crown. In some cases a considerable amount of mucilage appears in the cavity, and this apparently arises from the breaking down of the parenchyma cells. Although different in position, it recalls the slime-cavity or parichnos present in *Lepidodendon*, and in recent plants such as *Lycopodium* and some species of *Isoetes*.

The procambial strands, or desmogen, appear to originate in the leaves; passing through the periblem, or young cortex, they fuse into a sheath round the lower part of the parenchymatous cone which forms the medulla above the crown.

(*c*) *The Crown.*

This at maturity is a well-differentiated structure which separates the mature portion of the last year's shoot from the young tissues of the bud, and is pierced only by the procambial strands coming from the leaves.

In shape and character the crown may be described as a layer of thick-walled cells extending over the base of the growing tissues of the bud, and extending downwards on the inner side of the vascular ring for a short distance and upwards into the innermost scales of the bud. Its shape is shown in Fig. 1 at *cr.* During the late summer or early autumn the medulla immediately below the roof begins to break down, leaving a cavity extending down to the lowest prolongation of the crown. This cavity persists, and can be recognized in nodes many years old with the central arch of the crown present as a thin, horny layer above.

In sections of fresh material taken in the winter with a carbon dioxide freezing microtome, the cells of the roof stand out distinctly from the rest of the tissue. The cell-walls are thick, quite colourless and clear, and contrast with the greenish tinge of the adjoining medulla and old cortex. These walls give no lignin reaction, and tests for cellulose with chlor-zinc-iodine are negative, and this applies likewise to all the meristematic tissue and young leaves above the crown. The tissues below the crown, on the other hand, give a positive reaction with this reagent. The procambial strands give a cellulose reaction for a short distance above the crown. The walls of the crown are unstained by methylene blue alone, but if treated with hydrochloric acid and alcohol for twenty-four hours they then stain deeply with methylene blue, showing them to be formed of some pectic substance. The cell cavities are irregular in shape, often being much elongated in a transverse direction with irregular extensions; in fact, the tissue can be best described as a thick layer of pectic substance with irregular cavities. These irregular extensions of the cell cavities suggest the presence of intercellular communications.

Sections of fresh material were cut in the carbon dioxide freezing microtome and immediately transferred into 1 per cent. osmic acid for fixing. The material was then treated by the method given by Strasburger (3). Excellent preparations were obtained by this method, the structure being illustrated in Fig. 3.

The crown at maturity is a structure of pectic substance which completely divides off the older parts of the stem and the tissues of the bud, the only communication being by the procambial strands, in which lignification does not begin until a level just below the lowest extension of the crown is reached.

During the anatomical work some observations were made on the entry of eosin solution into the vascular system. One-year shoots were cut at various times in January and the cut end placed in eosin. Sections were taken forty-eight hours after with the carbon dioxide freezing microtome. It was found that eosin was conducted up the xylem strand as far as lignification extended, i. e. as far as the base of the crown. Slight infiltration had taken place into the disorganizing parenchyma cells just below and at the sides of the cavity. No penetration occurred in the crown cells or in any tissue of the bud above the crown. All the buds obtainable in a fresh condition were treated in this way, and the uniformity of the results suggests that water is prohibited from entering the dormant bud. According to Priestley (4) the meristematic cap in roots prevents the passage of water under a pressure of two atmospheres, but a similar condition of things does not exist in the stems of *Vicia Faba, L.*, and *Phaseolus vulgaris, L.* In the case of winter-buds with a long period of dormancy there must be some factor inhibiting the entry of water. The elongation of the bud begins during May, and by June considerable elongation has taken place and the young leaves are beginning to show. The actual dates show considerable variation, corresponding to the great differences in habitat of the tree. In Fig. 4 is shown the condition in material collected near Edmonton in early June 1923. The bud scales are not yet completely open, but the axis shows considerable elongation, due to the activity of the meristematic cells just below the initial cells at *i. c. pl.* (Fig. 2). The old crown is seen at *cr*^{1}., and there is some thickening of the new meristematic cells at *cr*^{2}., marking the position of the crown for the next winter's bud. The lateral and downward extensions are not yet developed. Lignification of the vascular strands now stretches as far as *lig.* (Fig. 4), and the strands passing in from the young leaves are also lignified towards the base of the bud. The subsequent elongation of the axis is due to the irregular divisions of the cells and their growth in size.

Picea Engelmannii, Engelm.

Engelmann's spruce is confined to Western Canada, and its distribution extends from about 8,000 feet in the Eastern Rockies to near sea-level at the Pacific coast.

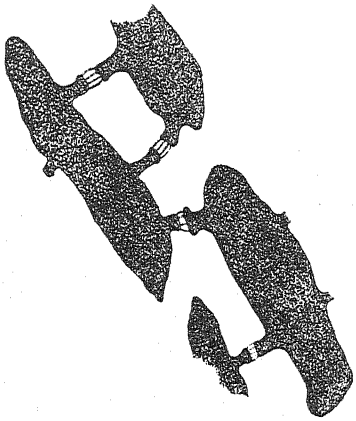

FIG. 3. *Picea canadensis*, January. Cells of crown treated to show protoplasmic intercommunication. Camera lucida, Zeiss $\frac{1}{12}''$, oil-immersion obj.

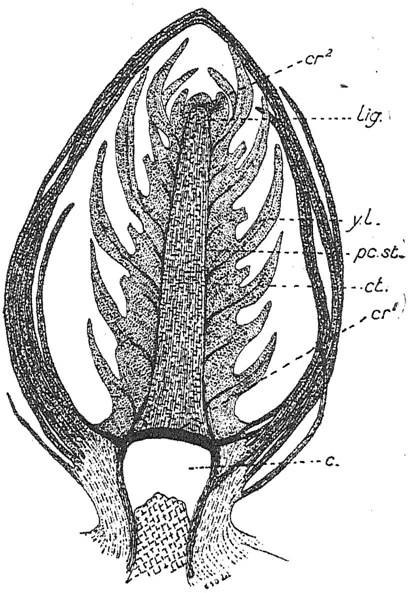

FIG. 4. *Picea canadensis*, June. Lateral bud. *cr*¹., crown of previous year; *cr*²., crown just arising at end of shoot; *pc. st.*, procambial strand; *y. l.*, young leaves; *ct.*, young cortex; *c.*, cavity; *lig.*, level of lignification.

The bud is somewhat larger and more massive in structure than *P. canadensis*, but presents similar features as regards relative position of cavity, crown, and meristematic tissue.

(*a*) *Growing point.* The pleurome arises from a group of initial cells (Fig. 5, *i. c. pl.*) clearly differentiated from the periblem in size, shape, and density of contents. The initial cells give rise to a thin layer of rapidly dividing cells below, which pass into iso-diametric, regularly arranged cells of the young medulla (Fig. 5, *pl.*). The dermatogen appears to be more sharply differentiated from the periblem than in the preceding species. By periclinal and anticlinal divisions the dermatogen gives rise to the primordia of the leaves seen in Fig. 5, *b*. The formation of the procambial strands or desmogen is represented by the outer, regularly arranged cells of the pleurome strand.

(*b*) The crown is relatively thicker and more massive than in the white spruce, and its lateral extensions stretch farther into the inner bracts. The lumen of the cells is smaller, but protoplasmic intercommunication occurs between the cells. The reactions of the wall are similar to *P. canadensis*.

Pseudotsuga Douglasii, Carr.

Douglas Fir occurs in the sub-alpine zone of the Rockies, and although occurring westward from the foot-hills of these mountains, it only reaches its greatest development at the Pacific coast. The general structure of the bud is similar to that of *Picea*.

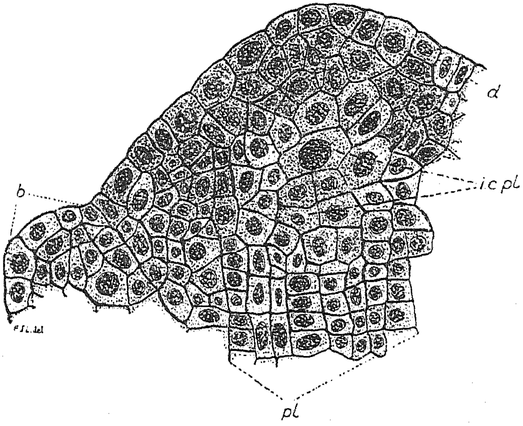

FIG. 5. *Picea Engelmanni*. Growing point of terminal bud, September. *pl.*, pleurome; *b.*, formation of leaf; *i. c. pl.*, initial cells of pleurome; *d.*, dermatogen.

The same differentiation of the growing point can be observed, the pleurome arising from a group of initial cells. The crown is less massive in structure, the downward prolongation is not so marked, and the transverse portion not quite so deep.

The cavity in the medulla below the crown forms later, and is not nearly so extensive. The old medulla is traversed by transverse bands of sclerotic cells, but consists of parenchyma only for some distance below and above the crown.

The growing point is shown in Fig. 6, the initial cells of the pleurome being distinguished as a group of about nine large cells giving rise to a narrow meristematic layer below, the procambial strands of the lower part of the bud being continuous with the regularly arranged row of cells at *pc. st.*

Larix Lyallii.

This tree is confined to high altitudes in the Rocky Mountains and Selkirks, being found at elevations of 6,800 to 7,800 ft. on ledges and slopes, often forming scattered woodland or parkland amongst alpine meadows. The growing season is short, the leaves usually not unfolding till late in June or early July, although snow may remain on the ground until the end of July. The leaves fall at the end of September.

The leaves are borne in fascicles, on short lateral branchlets which vary from 0·5 cm. to 1·5 cm. in length, according to age. In this species the branchlets produce leaves for about eight to nine successive years, and remain on the branches in a partly decayed condition for some years after leaf-bearing has ceased.

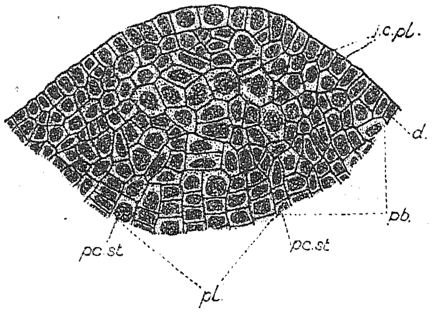

FIG. 6. *Pseudotsuga Douglasii.* Apex of bud showing differentiation of meristem in late September. *i. c. pl.*, initial cells of pleurome; *d.*, dermatogen; *pb.*, periblem; *pl.*, pleurome; *pc. st.*, row of cells giving rise to the procambial strands.

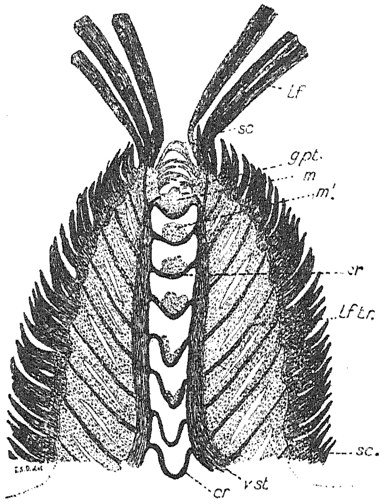

FIG. 7. *Larix Lyallii.* Lateral branchlet, July. *lf.*, leaves; *g. pt.*, growing point; *m.*, medulla; *m*¹., remains of medulla of previous year; *cr.*, crowns of successive years; *sc.*, scales; *v. st.*, vascular strand; *lf. tr.*, leaf-traces.

The structure of the branchlet and the terminal bud is illustrated in Fig. 7. The general characters of the bud as regards the breaking down of the medulla and the development of a crown are similar to the spruce, but owing to the deciduous habit of the tree and the small amount of growth of the lateral branchlets each year the relative position of the structures appears to be different. Owing to the slight amount of elongation every year the successive crowns fuse with each other.

The exterior of the branchlet is covered with the scars of old scales (*sc.*), covering the bud during the resting period. The vascular ring is broad at the base of the branchlet and represents seven years' growth. The length of the branchlet in Fig. 7 was 0·5 cm. Each year's growth is represented by a crown (*cr.*) of thickened cells, somewhat similar to those forming the crown in the spruces. The transverse portion of each crown is fused by a downward prolongation with the one of the former year, so that a continuous and irregular cylinder of thick-walled cells occurs inside the vascular ring with curved cross connexions at the point of bud production each year. There is no lateral extension of the crown as in the other genera described.

The medulla begins to break down soon after the bud elongates, and persists as a small mass of shrunken tissue (m^1.) above each successive crown.

When the bud is mature the crown consists of a few layers of thick-walled cells, which are sharply marked off from the thin-walled, delicate meristematic tissue of the growing point. The downward prolongations are not more than two cells in thickness transversely.

The cell-walls of the crown give negative tests for lignin and cellulose. No middle lamella can be seen with ordinary reagents. They stain deeply with corallin-soda. The tests for pectic substances are positive, and good reactions are given by the following reagents:

Treatment with hydrochloric acid and alcohol for twenty-four hours and subsequent staining with methylene blue gives a brilliant blue coloration to both the cell-walls and cell contents of the crown, whereas the rest of the cell-walls are only faintly coloured and the cell contents not at all. The cell contents of the crown, particularly, respond in a marked degree to this reaction, the contents being strongly vacuolated and filled with a granular substance coloured a bright blue.

Chlor-zinc-iodine gives a slight cellulose reaction of the wall adjacent to the lumen; this reaction is greatly intensified when the section has been previously treated with HCl. The middle portion of the cell-wall gives no reaction with chlor-zinc-iodine.

The meristematic tissue above the crown does not give a cellulose reaction, but responds in the same way to the positive tests given above for the thick-walled cells of the crown.

The procambial strand immediately above the crown gives a good cellulose reaction.

The tests point to the tissue of the crown and the meristematic region above (except the procambial strands) having walls of a pectic nature and not cellulose. Further progressive changes take place in the walls of the crown cells during late spring after the buds begin to elongate. These cells then gradually lose their outline, and the walls become shrunken and brown in colour. During the winter resting period the thick walls of the crown show excellent protoplasmic intercommunication from cell to cell, as in *Picea*, *Pseudotsuga*, and *Abies*. As far as our preparations show there is no differentiation at the growing point, thus agreeing with the observations of

Koch (2). The relation of the leaf primordia to the cone of meristematic tissue at *g. pt.* (Fig. 7) is entirely different to the genera already described. The primordia, instead of arising on the sides of the conical mass of meristematic tissue, arise from the tissue at the base. The apical region is cup-shaped, with a small conical mass of meristematic tissue situated on the floor of the cup, and giving rise to the medulla, procambial strands, and crown. The lower part of the cup produces fascicules of leaves, and the upper part scale leaves. Growth takes place by an expansion of the sides of the upper part of the cup with an upward growth of the floor. The disorganization of the medulla above the crown occurs early in the growing season.

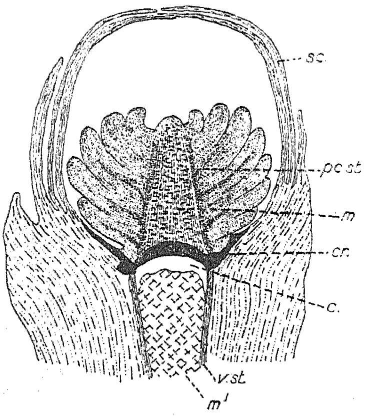

FIG. 8. *Abies lasiocarpa.* *sc.*, scales; *pc.st.*, procambial strands; *m.*, m^1., medulla; *cr.*, crown; *v.st.*, vascular strand; *c.*, cavity.

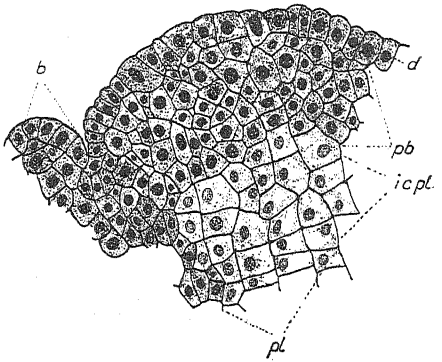

FIG. 9. *Abies lasiocarpa*, September. Apical region of bud with scales removed. *pl*, pleurome; *b.*, young leaf; *d.*, dermatogen; *pb.*, periblem; *i.e.pl.*, initial cells of pleurome.

Abies lasiocarpa (Hook.), Nutt.

Our examination of the bud was confined to material collected in September and November. The mature bud is larger in diameter and shorter than in the spruces examined, and although the same structures are present the relative development shows some differences.

As shown in Fig. 8, the young leaves are more fully formed in the late summer, and the lateral extensions of the crown pass out farther into the inner bracts, while the downward extension is absent and the whole bud is shorter and larger in diameter.

The general structure is illustrated in Fig. 8. The differentiation at the meristem of the growing point is well seen in all our longitudinal median sections. In Fig. 9 the initial cells of the pleurome are seen to occupy a comparatively large area (*i. c. pl.*), the cells being larger and less dense in contents than the outer periblem and dermatogen cells (*pb.*, *d.*). The beginning of the pleurome is shown at the bottom of the drawing. Both periclinal and anticlinal divisions occur in the dermatogen, and appear to be more frequent in most of our preparations at the extreme apical region than elsewhere, except at the point of origin of leaf primordia. The cortex is

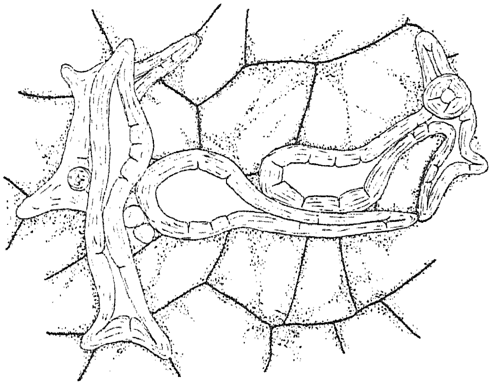

FIG. 10. *Abies lasiocarpa.* Branched sclereides in cortex immediately below the lateral extension of the crown.

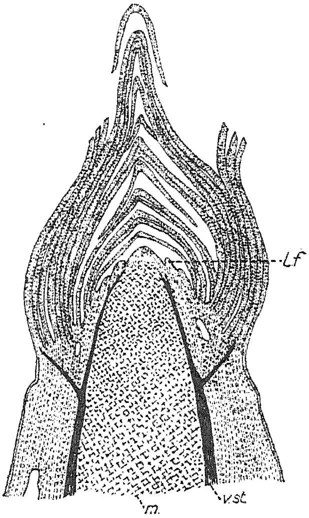

FIG. 11. *Pinus Murrayana*, September. *l. f.*, young leaf; *v.st.*, vascular strand; *m.*, medulla.

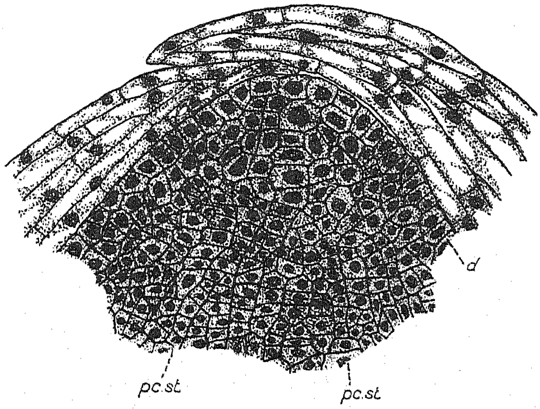

FIG. 12. *Pinus Murrayana.* Growing point showing no distinct differentiation in the meristematic layers. *pc. st.*, beginning of procambial strands; *d.*, dermatogen.

composed of lacunar parenchyma, and at the level of the crown and immediately outside branched sclereides are numerous, and are shown in Fig. 10. Such sclereides are common in several of the genera described, and are situated in the inner cortical region or in the outer cortical region in the neighbourhood of the leaf-traces. The medulla is without the transverse bars of thickened lignified cells characteristic of the spruces and Douglas Fir.

Pinus Murrayana, Balf.

Material has been collected at various seasons of the year. The arrangement of tissues is similar to that of other species of *Pinus*, there being no indication of any structure suggesting a crown as found in the other genera, neither does the medulla break down to form a cavity. As shown in Fig. 11, the general arrangement of the bud is simple, the primordia of the leaves arising from the gradually tapering stem apex. As seen in Fig. 12, there is no distinction of the meristematic layers at the growing point, the bud being in all respects entirely different and much simpler in structure than the other genera described.

SUMMARY.

1. The comparative structure of buds of species of *Picea*, *Pseudotsuga*, *Larix*, *Abies*, and *Pinus* has been studied.

2. In *Picea*, *Pseudotsuga*, *Larix*, and *Abies* the tissues of the bud are divided off from the older parts of the stem by a thick-walled structure giving pectic reactions, consisting of a horizontal plate with lateral and vertical extensions which we have called the crown.

3. The medulla breaks down below the crown to form a cavity, and this, with the remains of the crown, persists and can be recognized in branches several years old.

4. In *Picea*, *Pseudotsuga*, and *Abies* the meristematic tissue at the bud apex is differentiated into a group of initial cells giving rise to the pleurome. The dermatogen and periblem are not clearly differentiated from one another.

5. In *Larix* the pleurome, periblem, and dermatogen arise from a common mass of meristematic tissue.

6. In *Pinus* no differentiation of the meristematic region occurs, the crown is absent, and no cavity is formed in the medulla.

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Danae, Ruscus, and Semele: A Morphological Study.

BY

AGNES ARBER, D.Sc., F.L.S.

(*Keddey Fletcher-Warr Student of the University of London.*)

With fifty Figures in the Text.

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1. INTRODUCTION.

THE morphological nature of the so-called *phylloclades*, which form the assimilating organs of the members of the Liliaceous tribe *Rusceae* (Kunth)—*Danae*, *Ruscus*, and *Semele*—has been the subject of much controversy, and an extensive literature has grown up around the question. But I have found, on working through this literature, that I have been unable, from the existing descriptions and figures, to arrive at a clear comprehension, either of the structural relations of the phylloclades, considered as members of the shoot, or of their anatomy. I think that this may be partly due to the fact that hitherto the morphology has been studied chiefly by means of naked-eye observations, and

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by hand sections of the parts of the fully grown plants; and, at maturity, the phylloclades are not only refractory to sectioning, but have undergone considerable torsion, and, owing to their own hypertrophy and the wilting of the axillant leaf, their position in the shoot scheme is no longer easy to determine. So it seemed to me that, before one could arrive at an opinion about the nature of the phylloclades, a necessary preliminary was to make a study of transverse and longitudinal sections through the young shoot apices of the three genera—in addition to a general examination of the structure at maturity. I have been able to undertake such a study through the kindness with which I have been supplied with material by the Director of the Royal Botanic Gardens, Kew; Commendatore Cecil Hanbury, F.L.S., and Mr. Joseph Benbow, of La Mortola, Ventimiglia; the Director and the Superintendent of the Cambridge Botanic Garden, and Mrs. J. J. Lister. I also wish to express my indebtedness to Mr. T. A. Dymes, F.L.S., who generously placed at my disposal the material and notes for an account of the seedlings of *Ruscus aculeatus*, L., which he communicated to the Linnean Society on February 3, 1921.

The following section of this paper contains my own observations on the morphology of the monotypic genera *Danae* and *Semele*, and the three species forming the genus *Ruscus*. In that section I use the term phylloclade in a purely descriptive sense, while deferring all consideration of the morphological nature of these organs to the third section. I hope, in a later paper, to study the 'needles' of the related genus *Asparagus*.

2. DESCRIPTION OF OBSERVATIONS.

(i) *Danae racemosa*, (L.) Mönch.

The genus *Danae* includes only the one species, *D. racemosa*, (L.) Mönch., sometimes called the Alexandrian Laurel, or Victor's Laurel, which is found in N. Syria, Transcaucasia and N. Persia. Its assimilating organs consist chiefly of phylloclades (*p.*), such as those shown in Fig. 1 A, and in Figs. 2 A and 3 A, B, p. 232. But it also produces two types of leaf, which we will consider before turning to the phylloclades. The seedling, and occasionally, also, the mature rhizome, produces ordinary foliage leaves[1] (4, 27), such as *f.* in Fig. 1 A, drawn from a plant from La Mortola. This leaf, whose anatomy is shown in the series of sections 1 B–E, had a short sheathing base, a long well-marked petiole (*pet.*), triangular in section (Fig. 1 C), and a limb with a definite midrib. This limb was supplied by a single series of normally orientated bundles; in the section drawn in Fig. 1 E there were stomates in the lower epidermis of the leaf, which was succeeded by two layers of assimilating tissue; while beneath the upper, stomateless epidermis, there were three layers of assimilating tissue.

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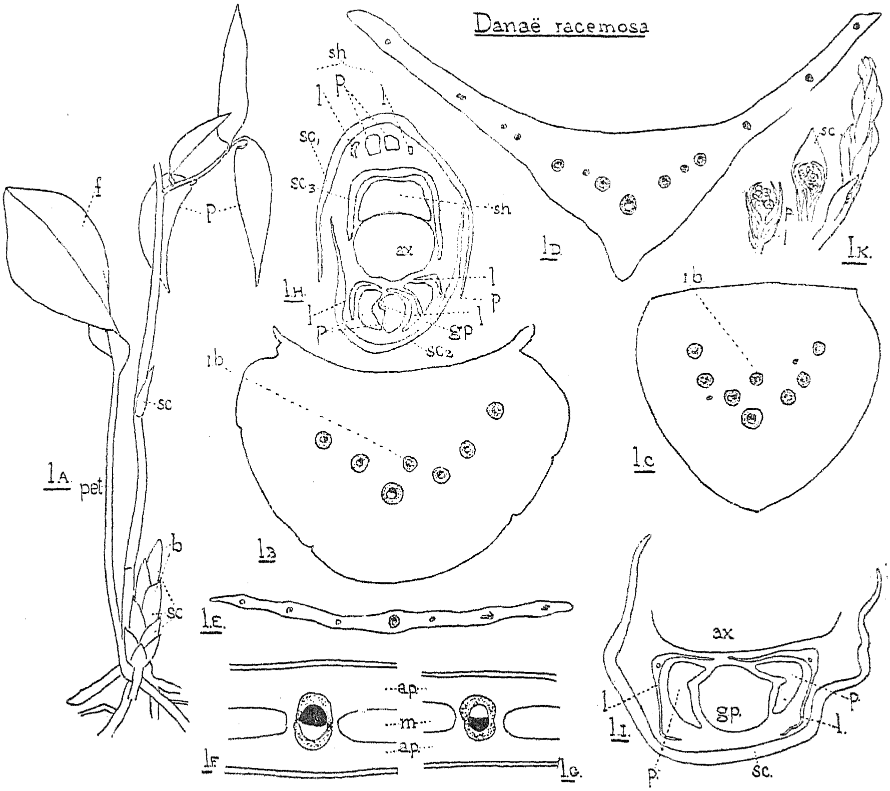
[1] Reinke (24) erroneously states that no such leaves exist in *Danae*.

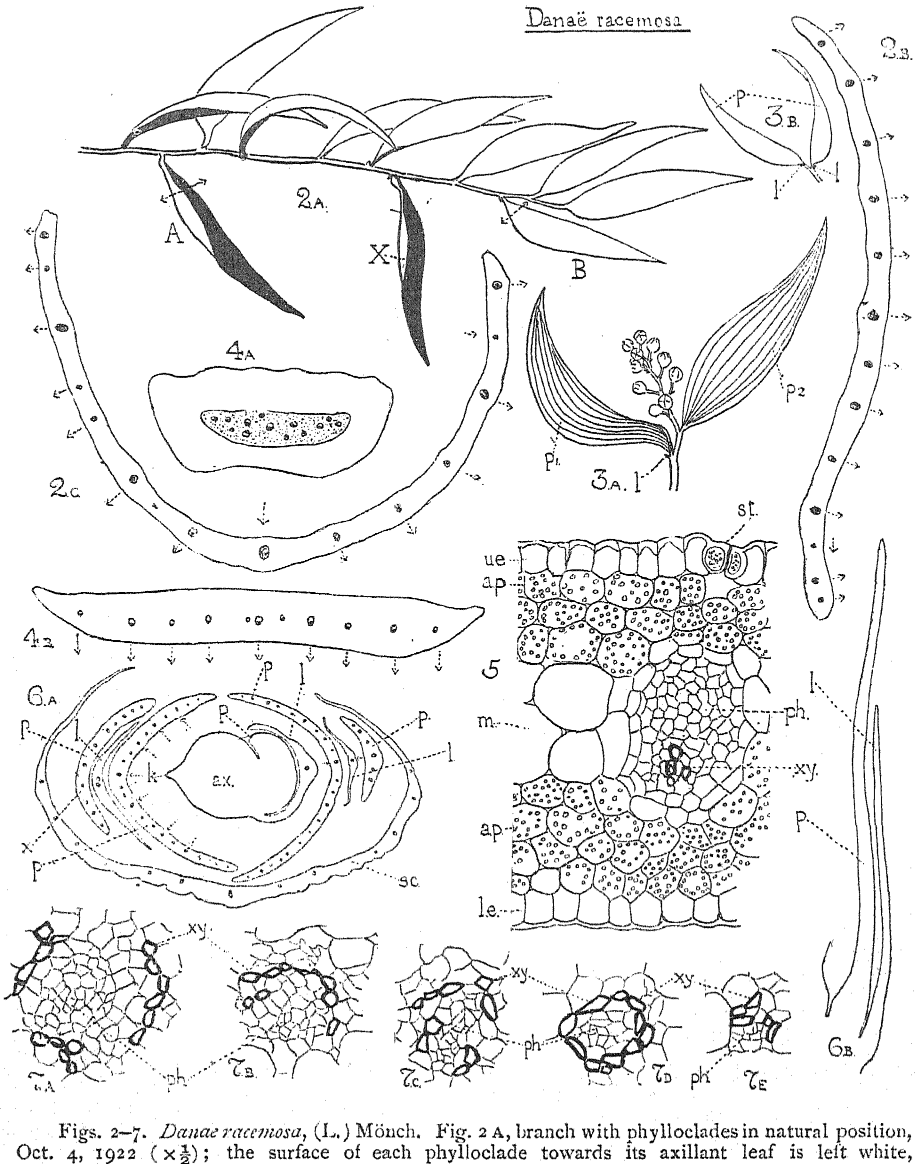
There is thus nothing peculiar in the general lines on which this leaf is constructed.

While normal leaves, such as that just described, are relative rarities



Figs. 1 A–K. *Danae racemosa*, (L.) Mönch. (Where sections are represented in this and later figures, xylem is indicated in black; phloem, white; and fibres, dotted). Fig. 1 A, part of a plant from La Mortola, Ventimiglia, March, 1921 (× ½); *b.*, winter-bud; *sc.*, scale-leaves; *f.*, primary foliage leaf with long petiole, *pet.*; *p.*, phylloclades. Figs. 1 B–E, transverse sections through leaf *f.* in Fig. 1 A; *i.b.*, bundles with xylems turned downwards (× 14). Fig. 1 B, top of short basal sheath; Fig. 1 C, high in petiole; Fig. 1 D, base of limb; Fig. 1 E, near apex of limb. Fig. 1 F, small part of Fig. 1 E (or similar section) to show single vein of foliage leaf (× 77) for comparison with Fig. 1 G, which shows, on same scale, a single vein from near apex of one of the phylloclades, *p.*, in Fig. 1 A; *a.p.*, assimilating parenchyma; *m.*, colourless large-celled mesophyll. Fig. 1 H, transverse section from microtome series through central part of bud, such as *b.* in Fig. 1 A (× 23), including axis, *ax.*; three scale-leaves, $sc._1$, $sc._2$, $sc._3$, each subtending a bud. The bud, *sh.*, inside $sc._3$, is cut at a level below its appendages; the buds inside $sc._1$ and $sc._2$ show leaves, *l.*, with phylloclades, *p.*, in their axils; *g.p.*, growing apex. Fig. 1 I, bud in axil of scale-leaf, *sc.*, similar to the three buds shown in Fig. 1 G, but rather older. The growing axis of the bud, *g.p.*, has produced a pair of prophyllar leaves, *l.*, subtending phylloclades, *p.* Fig. 1 K, tip of bud, such as *b.* in Fig. 1 A, later in the spring (reduced); to the left, one of the axillary branches of this shoot with its terminal inflorescence is drawn from the adaxial side, with and without its axillant scale leaf (× 1).

in *Danae*, a reduced type—the scale-leaf—is produced in abundance. In the winter bud *b.* in Fig. 1 A, only scale-leaves are visible. Figs. 1 H and 1 show the details of such a bud when sectioned. The scale-leaves



Figs. 2–7. *Danae racemosa*, (L.) Mönch. Fig. 2 A, branch with phylloclades in natural position, Oct. 4, 1922 (×½); the surface of each phylloclade towards its axillant leaf is left white, and surface away from axillant leaf marked black. Fig. 2 B, transverse section, at level of arrow, of phylloclade B, which is held edgeways (×14); the dotted arrows indicate direction in which xylem of bundles faces. Fig. 2 C, transverse section, at level of arrow, of phylloclade A, which is held in unusual position, with surface away from axillant leaf uppermost (×14). Fig. 3 A, shoot bearing leaf *l.*, in whose axil phylloclade *p.*$_1$ arises; a second phylloclade, *p.*$_2$, whose axillant leaf is invisible; a terminal raceme of young fruits, still enclosed in persistent perianths (×½). Fig. 3 B, end of sterile shoot, with two phylloclades, *p.*, in axils of leaves, *l.*: no terminal phylloclade (×½). Figs. 4 A and B, transverse sections of a phylloclade (×23). Fig. 4 A, stalk; Fig. 4 B, near base of expanded region. Fig. 5, small segment of transverse section of a young phylloclade (×318); fibres of bundle-sheath still thin-walled; *xy.*, xylem; *ph.*, phloem; *st.*, stomates; *u.e.*, upper epidermis; *l.e.*, lower epidermis; *a.p.*, assimilating parenchyma; *m.*, colourless mesophyll. Fig. 6 A, transverse section of young bud (May 24, 1922) in axil of scale-leaf,

sc., which are in two opposite ranks, each subtend an axillary shoot, *sh.* This shoot, again, bears two lateral series of leaves (*l.* in Figs. 1 H and I), each with a young phylloclade, *p.*, in its axil. The relation of the leaf and phylloclade is shown in radial longitudinal section in Fig. 6 B, p. 232. Fig. 1 K, p. 231, represents a later stage of development of such a bud as *b.* in Fig. 1 A; the internodes between the scale-leaves have elongated, and the shoots in the axils of those in the distal region are beginning to reveal the racemes of flower-buds in which many of them terminate. A shoot with two mature phylloclades and a group of young fruits is shown in Fig. 3 A, p. 232; the fact that the inflorescences are independent of the phylloclades is a point in which *Danae* differs from *Ruscus* and *Semele*. In those shoots of *Danae* which are not reproductive the growing point aborts, after the production of a certain number of scale-leaves and phylloclades. The apex of such a shoot is drawn in Fig. 3 B; it will be seen that the topmost phylloclade—like all the others—arises in the axil of a scale-leaf. I have not, in this species, ever seen terminal phylloclades, such as occur at the ends of the shoots of *Ruscus*.

The shoot at the stage of Fig. 1 K, p. 231, may be compared with the section shown in Fig. 6 A, p. 232, which is cut from a bud slightly older than those shown in Figs. 1 H and I. The vascular tissue is differentiating. The leaves, *l.*, which subtend the phylloclades, have a well-marked midrib, which gives a keeled form, *k.*, to the transverse section of the axis. At this young stage, these leaves are usually, though not always, supplied by one bundle only; this bundle shows considerable irregularity, with a tendency to an amphivasal form—the xylem surrounding the phloem. In Figs. 7 A–E one of these bundles is followed upwards through its leaf, which is that axillant to the phylloclade marked × in Fig. 6 A. The bundle is concentric at the extreme base, and at first retains this form (Fig. 7 A), but subsequently becomes collateral (Fig. 7 B), then resumes its concentric form (Figs. 7 C and D), and finally, before it dies out near the leaf apex, becomes collateral again (Fig. 7 E).

The vascular system of the young phylloclades is of a different type; they have no obvious median strand, and their most noticeable feature, at this stage, is the fact that their bundles are orientated in the opposite sense to that of the axillant leaf. The structure of the phylloclade is, however, best understood from older material; Figs. 4 A and B show transverse sections of the stalk-like base of the mature phylloclade, and of

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sc.; its axis, *ax.*, bears two rows of leaves, *l.*, subtending phylloclades, *p.* The leaves in whose axils the two outermost phylloclades arise do not reach to this level. The keel, *k.*, of the axis corresponds to the midrib of the next leaf above (× 14). Fig. 6 B, radial longitudinal section through leaf *l.* with phylloclade *p.* in its axil, from microtome series through bud, May 24, 1922 (× 14). Fig. 7 A–E, series of transverse sections (× 318) to show history of the single bundle of the leaf in whose axil the phylloclade marked with a × in Fig. 6 A arises; Fig. 7 A, near leaf base; Fig. 7 E, near leaf apex.

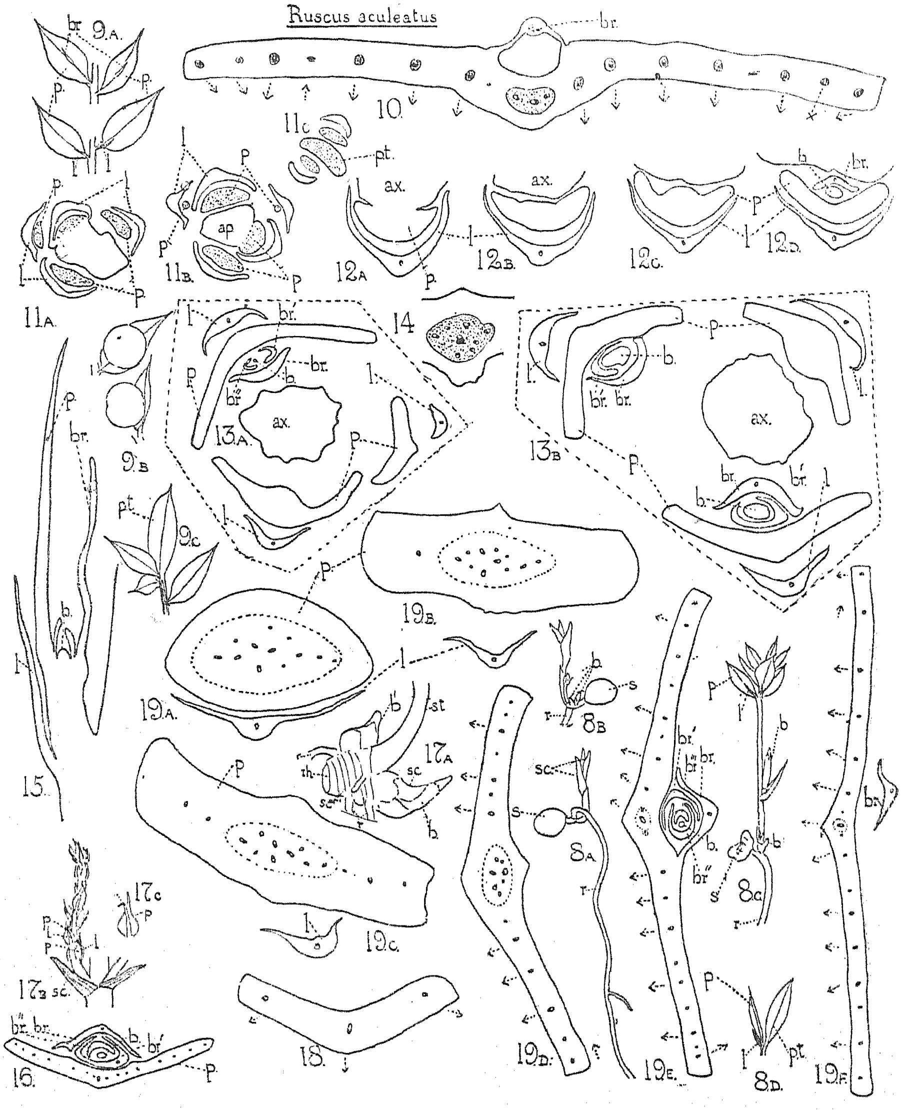
the proximal region of the expanded limb. In the stalk, the bundles are grouped into an irregular, flattened central cylinder, while in the limb they form one series with the xylem directed downwards. Fig. 5, p. 232, indicates the detailed structure of a small segment of a phylloclade, including one bundle, whose phloem lies towards the upper surface. The peculiar character of the bundle orientation tends to be obscured at maturity, because of the way in which the phylloclades hold themselves. An inspection of a clump of Victor's Laurel shows that the phylloclades have a strong tendency to place themselves edgeways, by means of basal torsion. In Fig. 2 A, p. 232, which is a sketch of the distal part of a branch in its natural position, the surfaces of the phylloclades away from the axillant leaves are indicated in black, while the sides towards the axillant leaves are left white. It will be seen that only A and X hold themselves with the side away from the axillant leaf uppermost. Fig. 2 B shows a transverse section of one of the vertical phylloclades, B, at the level marked with an arrow in Fig. 2 A. There are thirteen bundles; one small strand lies sideways, but all the rest have their xylems directed to the right, i.e. towards the surface which was originally turned to the axillant leaf, though now twisted away from it, through an angle of 90°. In the comparatively rare cases (e.g. A in Fig. 2 A) in which there is no torsion of the base of the phylloclade, and in which the side towards the axillant leaf thus remains downwards, the phylloclade, instead of being flat, curves up at the margins into an almost trough-like form, as though endeavouring to arrive, by other means, at a result approximating to that normally reached by the twisting of the base. Fig. 2 C shows a transverse section of such a phylloclade as that marked A in the sketch, cut at the level of the arrow. There are fifteen bundles, all of which have their xylem on the lower side, i.e. towards the axillant leaf.

(ii) *Ruscus aculeatus*, L.

The genus *Ruscus* includes three species, one of which, *R. aculeatus*, L.—Butcher's Broom or Knee Holly—extends from the Mediterranean region, through France and Belgium, to Great Britain. Neither in this plant, nor in the other two members of the genus, have petiolate foliage leaves, comparable with those of *Danae* and *Semele*, ever been recorded; de Candolle, who figured the seedling nearly a century ago (9, vol. ii, Pl. 49), pointed out that the axis bore, from the first, only scale-leaves, and subsequently, scale-leaves with phylloclades in their axils. Two seedlings are shown here in Figs. 8 A–D, p. 236. In the mature plant the rhizome, if examined in the early spring, will be found to bear winter-buds clothed with scale-leaves, such as *b.* and *b'.*, Fig. 17 A. Later the bud axis elongates, and branches are developed in the axils of the upper scale-leaves (Fig. 17 B); these branches bear narrow pointed leaves, *l.*, with phylloclades, *p.*, in their axils (Fig. 17 C, p. 236). The relation to one another of the developing leaves (white) and phylloclades (dotted) is shown in Figs. 11 A–C, which represent sections through a lateral bud gathered in May. The leaves, instead of being distichous, as in *Danae*, are arranged spirally. At this very early stage, the phylloclades are small and insignificant in comparison with their axillant leaves, and have not produced any appendages; but in Figs. 12 A–D a slightly older phylloclade is shown, which, though its apex does not yet reach up to the top of its axillant leaf, has developed so far as to give off a bract and bud on the adaxial side (*br.* and *b.*, Fig. 12 D). Fig. 15 may be compared with Fig. 12; it represents a radial longitudinal section through a still older phylloclade, with its bract and bud. Figs. 13 A and B, 16, and 19 E illustrate a curious feature of the buds borne on the phylloclades—namely, that the arrangement of the bracteoles is not always the same, but may conform to one of two distinct types. In Figs. 13 A and 19 E the bracteoles, *br'.* and *br''.*, are strictly transversal, i. e. at right angles to the bract *br.*, whereas in Figs. 13 B and 16 they are obliquely placed. The idea suggests itself that possibly one of these arrangements may characterize the female flower-bud, and the other the male, but I have not succeeded in testing this notion, since the bracteoles are shrunk and wilted by the time the flower is developed.

The mature phylloclades, with their axillant leaves and bracts, are shown on a reduced scale in Fig. 9 A, while a fruiting example is drawn in Fig. 9 B. Fig. 9 C illustrates the fact—which can also be seen in the seedling, Fig. 8 D—that the last phylloclade borne on the axis does not arise in the axil of a scale-leaf, but is terminal and slightly decurrent (6 and 15). The same feature is shown in a section of a young bud in Fig. 11 C; in every case in which a terminal phylloclade is included in a drawing, it is marked *p. t.*

Though there is no torsion in the youngest stages, the phylloclades are finally held more or less in the vertical plane; the way in which this attitude is attained is demonstrated by the series of sections in Figs. 19 A–F, which are cut, from the base upwards, through an immature phylloclade. Fig. 19 A is the stalk of the phylloclade, which in Fig. 19 B is becoming flattened, and in Fig. 19 C is beginning to twist. In Fig. 19 D the vertical is approached, and it is finally reached in Fig. 19 E. The midrib, from the base up to the level of the bract, is traversed by a cylinder including several bundles, but by the time the bract and bud have received their vascular supply the radial structure is lost, and we are left with only a median bundle and one or two laterals in the midrib (Figs. 19 E and F). The midrib of the sterile phylloclade may also include a group of bundles (Fig. 14). In Figs. 19 D–E the arrows indicate the direction in which the xylem points in the expanded part of the phylloclade. It will be seen that, with rare exceptions, the xylem is, as in *Danae*, directed towards the axillant



Figs. 8–19. *Ruscus aculeatus*, L. Figs. 8 A–D, seedlings grown by Mr. T. A. Dymes, Feb. 21, 1923. Figs. 8 A and B, young subterranean seedling, in which only scale-leaves, *sc.*, have developed, viewed from the two sides (× ½); in Fig. 8 B three lateral buds, *b.*, are visible; *s.*, seed. Figs. 8 C and D, older seedling bearing phylloclades, *p.*, in axils of scale-leaves, *l.*; two lateral buds, *b.*; seed, *s.*, shrunken as compared with Figs. 8 A and B; *r.*, root (× ½). Fig. 8 D, two uppermost phylloclades of 8 C, one in axil of leaf, while the other, *p.t.*, is terminal (× 1). Fig. 9 A, two successive phylloclades, *p.*, of mature shoot, viewed from the two sides; *l.*, axillant leaves; *br.*, bracts borne on phylloclades (× ½). Fig. 9 B, two views of a phylloclade bearing a fruit, Feb. 1923 (× ½). Fig. 9 C, end of shoot showing terminal phylloclade, *p.t.* (× ½). Fig. 10, transverse section of phylloclade at level of its junction with bract, *br.* (× 14). Arrows show directions in which xylem groups of bundles point; bundle marked with a × is amphivasal; axillant leaf would lie to foot of page. Figs. 11 A–C, transverse sections from series through lateral bud, May 13, 1922 (× 23); *l.*, leaves (white); *p.*, phylloclades (dotted). Fig. 11 C shows further development of *ap.*, growing apex, in 11 B, with the formation of two leaves subtending phylloclades, and a third phylloclade which is terminal, *p.t.* Figs. 12 A–D, series of sections through leaf, *l.*, with phylloclade, *p.*, in its axil, May 13, 1922 (× 23);

leaf. Fig. 18 shows a transverse section near the tip of a young phylloclade, in which the bundles, as they approached the apex, were reduced to three. Fig. 10 is a sketch of a transverse section of an older phylloclade, at a level at which the bract was still attached, though the bud had become free, and fell away when the section was cut; at this stage the bundle-sheaths had become fibrous. The bract has its bundle orientated like those in the phylloclade. As in the younger phylloclade represented in Fig. 19, the midrib above the bract has lost its radial structure.

(iii) *Ruscus Hypoglossum*, L.

The South European *Ruscus Hypoglossum*, L., has leafy-looking phylloclades, which are on a larger scale than those of *R. aculeatus*, and which, though firm in texture, are not stiff and spiny. Figs. 20 A–C, p. 238, show the structure of two seedlings, after a year from sowing. The one which was more advanced bore scale-leaves, *sc.*, followed by a phylloclade, *p.*, in the axil of a leaf, *l.*, and a second phylloclade, *p.t.*, which was not in the axil of a leaf, but was terminal to the axis. The shoots of the mature plant end in the same way (Fig. 20 D).

The bract borne on the phylloclade of *R. Hypoglossum*, instead of being delicate and ephemeral, as in the other two species of *Ruscus*, is of the same consistency as the phylloclade itself; it may sometimes reach a considerable size (var. *macroglossa*, Fig. 20 E). The bract of the normal form is shown in section in Figs. 21 H and I, 22 B and C. *R. Hypoglossum* is generally described as producing its bracts on the adaxial side of the phylloclades, like *R. aculeatus*, but in the fresh material from La Mortola, which I was able to examine, I found great variety in this respect. The phylloclades are irregularly arranged—sometimes spirally, and sometimes two or three together at a node—and even at a single node we may find

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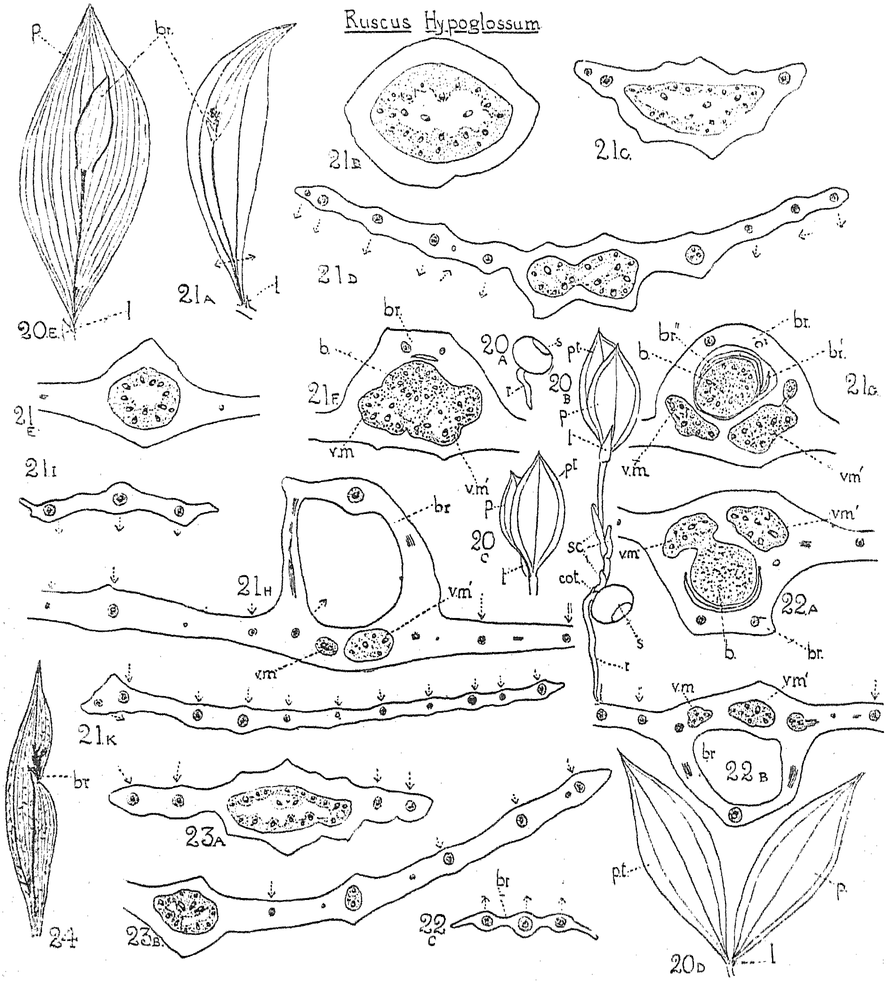
Fig. 12 A shows phylloclade not yet detached from axis, *ax.*, while Fig. 12 D shows bract, *br.*, and its axillary bud, *b.*, on side of phylloclade remote from axillant leaf. Figs. 13 A and B (enclosed in dotted lines), transverse sections through buds, June 2, 1922 (× 14), in each case showing axis, *ax.*, and three leaves, *l.*, with phylloclades, *p.*, in their axils. One phylloclade in each case is cut just above the attachment of the bract, *br.*, which arises on its adaxial surface, and subtends bud, *b.* The buds differ in that that in Fig. 13 A has two first leaves, *br′.* and *br″.*, which are lateral and opposite, while those in Fig. 13 B have one larger, obliquely placed first leaf, *br′.* Fig. 14, transverse section of midrib of a sterile phylloclade (× 23). Fig. 15, radial longitudinal section of leaf *l.* with phylloclade *p.* in its axil, bearing bract, *br.*, and bud, *b.*; June 2, 1922 (× 14). Fig. 16, transverse section of phylloclade, *p.*, June 16, 1919, bearing bract, *br.*, in whose axil is the bud *b.*, bearing leaves *br′.* and *br″.* arranged as in the buds in Fig. 13 B (× 14). Fig. 17 A, end of rhizome, *rh.*, marked with scars of former scale-leaves, *sc′.*, and bearing roots, *r.*, and terminating in aerial stem, *st.* The two winter-buds, *b.* and *b′.*, clothed with scales, *sc.*, will develop into the shoots of the current year, Feb. 6, 1923 (×½). Figs. 17 B and C, part of young shoot later in the spring, probably May. Fig. 17 B, branch in axil of scale-leaf, *sc.*, bearing leaves, *l.*, and phylloclades, *p.* (×½). Fig. 17 C, leaf and phylloclade, *p.*, of Fig. 17 B (× 1). Fig. 18, transverse section near apex of a young phylloclade (June 16, 1919) to show reduction of bundles to three; axillant leaf would lie towards lower edge of page (× 23). Figs. 19 A–F, series of transverse sections from base upwards through leaf, *l.*, and its phylloclade, *p.*, June 16, 1919 (× 14). Figs. 19 C and D show the process of torsion that brings the phylloclade, *p.*, into the vertical position in Figs. 19 E and F. The arrows show direction of xylem in bundles. Fig. 19 E passes through the attachment of bract, *br.*, in whose axil the bud, *b.*, arises, bearing leaves, *br′.*, *br″.*, *br‴.* Fig. 19 F is above attachment of bract, *br.*



Figs. 20–24. *Ruscus Hypoglossum*, L. (small arrows indicate direction in which xylem groups of bundles point). Figs. 20 A–C, seedling structure, from seeds from La Mortola, sown March 20, 1922, drawn Feb. 16, 1923 (× 1). Fig. 20 A, seedling with only radicle, *r.*, protruding from seed, *s.* Fig. 20 B, seedling with cotyledon, *cot.*, three scale-leaves, *sc.*, followed by a leaf, *l.*, in whose axil is a phylloclade, *p.*, while the shoot ends in a terminal phylloclade, *p.t.* Fig. 20 C, view of *l.*, *p.*, and *p.t.* from the opposite side. Fig. 20 D, apical part of mature shoot (× ½) to show terminal phylloclade, *p.t.*, standing apparently opposite to a phylloclade, *p.*, in axil of leaf, *l.* Fig. 20 E, phylloclade, *p.*, bearing leathery bract, *br.*, on side away from axillant leaf, *l.*, Brit. Mus. Herb. (probably var. *macroglossa*) (× ½). Fig. 21 A, phylloclade (venation shown in less detail than in Fig. 20 E) bearing bract, *br.*, and remains of inflorescence on surface away from axillant leaf, *l.* (× ½). Figs. 21 B, C, D, E, H, I, K, anatomical structure of phylloclade drawn in Fig. 21 A. Figs. 21 F and G, supplementary stages from another phylloclade (all × 14). Fig. 21 B, near extreme base of phylloclade; Fig. 21 D, at level of arrow in Fig. 21 A; Fig. 21 C, intermediate. Fig. 21 E, midrib region higher up; ridge above vascular cylinder is first indication of bract. Fig. 21 F, section from another phylloclade at a slightly higher level, showing bract, *br.*; vascular system, *b.*, for bud axis; *v m.* and *v.m′.*, vascular masses destined for main veins of upper part of phylloclade. Fig. 21 G, same phylloclade as in Fig. 21 F, cut at a slightly higher level; bud now free, bearing two leaves, *br′.* and *br″*. Fig. 21 H, section from phylloclade in Fig. 21 A, cut near base of leathery bract; bud has fallen out; *v.m.* and *v.m′.* as in Fig. 21 F. Fig. 21 I, bract after detachment.

examples of both adaxial and abaxial buds. And abnormal cases occasionally occur in which the bract becomes marginal owing to the peculiar form of the phylloclade (Fig. 24, p. 238).

The vascular system of the phylloclades is more highly developed than in *R. aculeatus*. Figs. 21 B–I show the structure of the phylloclade, and the origin of the vascular system for the bract and lateral branch, in examples in which the bract occurs on the adaxial side, while Figs. 22 A–C illustrate the converse case, in which the bract is abaxial. It will be noted that the main veins, both below and above the exit of the bract, are supplied by vascular cylinders, instead of single bundles. Sections a little below the origin of the bract show the midrib with a ring of bundles embedded in a fibrous sheath (Fig. 21 E). Just at the base of the bract, the midrib branches into three parts (*v.m.*, *v.m′.*, and *b.*, Fig. 21 F), which are completely separated in Fig. 21 G (see also Fig. 22 A). The median mass, *b.*, which is circular in section and contains a ring of bundles, supplies the bud axis. The other two, *v.m.* and *v.m′.*, which each enclose a flattened and irregular ring of bundles, remain to supply the upper part of the phylloclade. In passing upwards, *v.m.* and *v.m′.* divide into small bundle groups, and in Fig. 21 K, which is near the apex of the phylloclade, the veins consist of single bundles. Throughout the phylloclade, those bundles which are not grouped into steles are placed with their xylem directed towards the axillant leaf.

The bract, whether it is on the upper or under surface, has its xylem turned towards the phylloclade (Figs. 21 I and 22 C).

It is not only the fertile phylloclades in which the veins have a radial structure; Figs. 23 A and B are sections of a phylloclade which bore no bract or bud, and it will be seen that the midrib and one of the lateral veins in Fig. 23 B are traversed by steles instead of single bundles.

(iv) *Ruscus Hypophyllum*, L.

Ruscus Hypophyllum, L., which extends from Madeira to the Caucasus, has a strong general resemblance to *R. Hypoglossum*, but differs from it in the fact that the bract borne by the phylloclade is fragile and membranous, instead of being tough and leathery. The only seedling which I have been able to examine (Fig. 25 A, p. 240) was one year old. It closely resembled that of *R. Hypoglossum*; scale leaves were followed by two phylloclades, one of which, *p.*, was subtended by a scale-leaf, *l.*, while the other was terminal, *p.t.*. The ends of the branches in the mature

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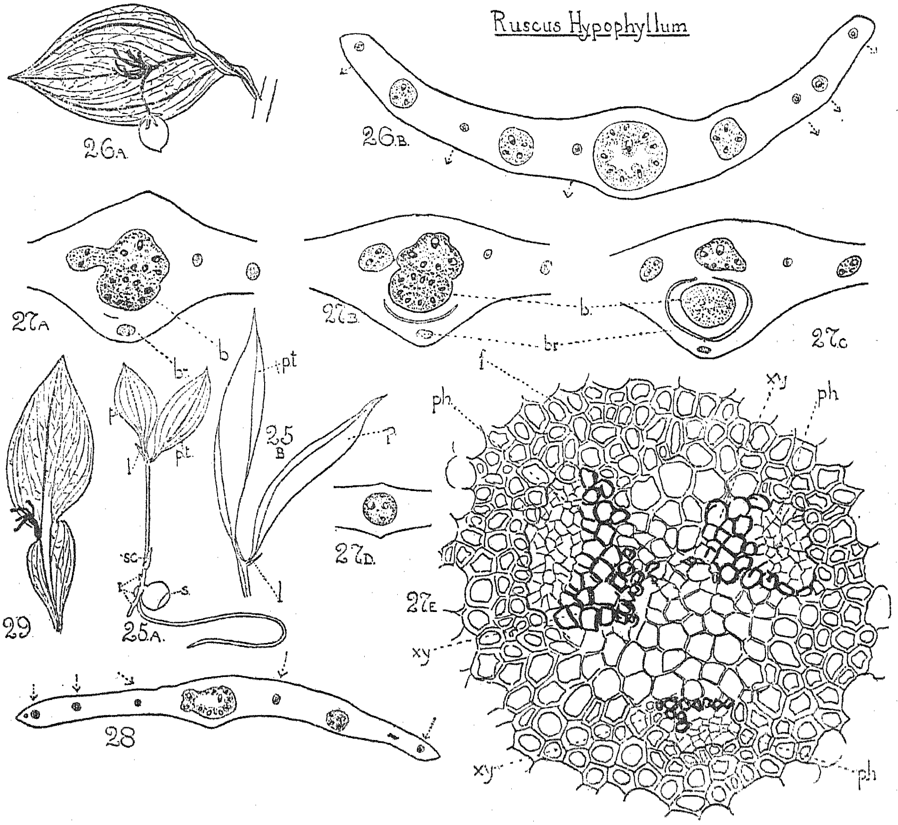
Fig. 21 K, section near apex of phylloclade. Figs. 22 A–C, successive sections (× 14) through bract-bearing region, *br.*, of another phylloclade, in which the inflorescence was on the side towards the axillant leaf; these sections correspond to Figs. 21 G, H, I. Figs. 23 A and B, transverse sections of a sterile phylloclade, near the base and a little higher (× 14). Fig. 24, an abnormal phylloclade (× ½), drawn from side away from axillant leaf. (Figs. 21–24, from material from La Mortola, March, 1922.)

plant also usually bear a somewhat decurrent terminal phylloclade (*p.t.*, Fig. 25 B).

In the fertile phylloclades the bract and inflorescence are generally situated on the surface towards the axillant leaf (Fig. 26 A), but this rule is by no means invariable, for the inflorescences sometimes occur on the upper side, and abnormal lobed phylloclades are also to be found, in which the inflorescence is marginal (Fig. 29). The vascular system of the phylloclade closely recalls that of *R. Hypoglossum.* Figs. 27 A–C show the origin of the vascular supply of the bud. As in *R. Hypoglossum*,



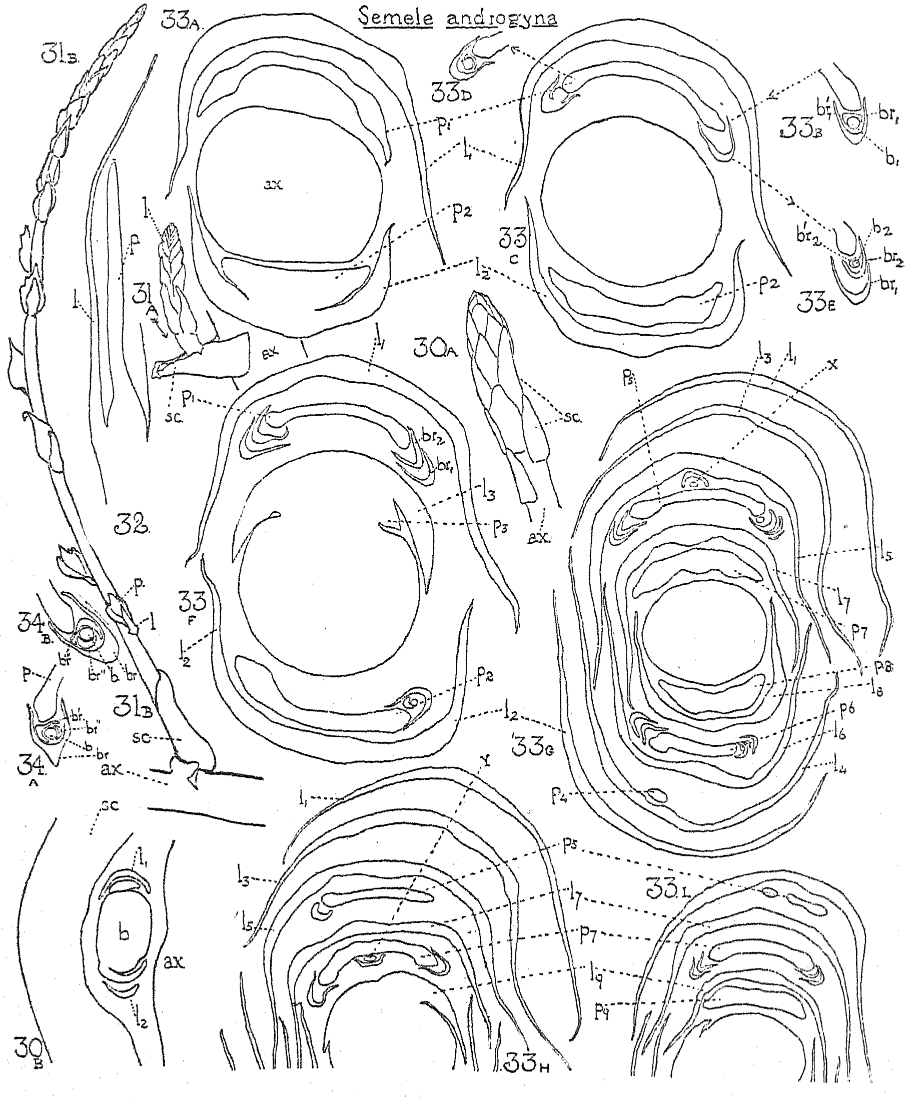
Figs. 25–29. *Ruscus Hypophyllum*, L. (material from La Mortola). Fig. 25 A, seedling ($\times \frac{1}{2}$); seed from La Mortola, planted March 20, 1922, drawn Feb. 16, 1923; *s.*, seed; *r.*, roots; *l.*, leaf with phylloclade, *p.*, in its axil; *p.t.*, terminal phylloclade. The main root, which should be vertical, bent to one side to economize space. Fig. 25 B, end of a mature shoot, to show terminal phylloclade, *p.t.*; *l.*, leaf ($\times \frac{1}{2}$). Fig. 26 A, phylloclade with remains of inflorescence and one fruit on side towards axillant leaf ($\times \frac{1}{2}$). Fig. 26 B, transverse section of a fertile phylloclade near the base ($\times 14$). Figs. 27 A, B, C, series of three sections of a phylloclade showing origin of bract, *br.*, and vascular cylinder of bud, *b.* ($\times 14$). Figs. 27 D and E, transverse section of midrib of phylloclade above inflorescence ($\times 14$ and 193); *xy.*, xylem; *ph.*, phloem; *f.*, fibres. Fig. 28, transverse section of sterile phylloclade not far from base ($\times 14$). Fig. 29, abnormal phylloclade with inflorescence at margin, flowers fallen ($\times \frac{1}{2}$).

the main veins both below the inflorescence (Fig. 26 B) and above its exit (Fig. 27 C, D, E) are radial in structure. This is true also of the main veins of the sterile phylloclade (Fig. 28). In both sterile and fertile phylloclades, those veins which consist of one bundle only have their xylems directed towards the axillant leaf (Figs. 26 B and 28).

(v) *Semele androgyna*, (L.) Kunth.

The monotypic *Semele androgyna*, (L.) Kunth, of the Canary Islands, differs from the other Rusceae in being a climbing shrub. In the spring, aerial axes clothed with scale-leaves (Fig. 30 A, p. 242) are sent out from the rhizome; they resemble gigantic shoots of *Asparagus officinalis*, L., and may grow to a great length before they branch. Each scale-leaf, *sc.*, has a bud in its axil, the two first leaves of which ($l._1$ and $l._2$ in Fig. 30 B) are placed transversely. Fig. 31 A shows the result of about one month's further development of a bud such as *b.* in Fig. 30 B, while Fig. 31 B was drawn from another shoot, later in the spring. The scale-leaf, *sc.*, in Fig. 31 B, has become torn at the base by the growth of its axillary branch, which is now seen to bear leaves, *l.*, in two alternating series, each with a phylloclade, *p.*, in its axil. In Figs. 33 A–I we can follow the development of leaves and phylloclades in the apical region of a branch, such as that drawn in Fig. 31 B, while Fig. 32 shows a median longitudinal section of a leaf, *l.*, with its phylloclade, *p.* In Figs. 33 B, C, D, and E, the history of the bracts and buds borne on the phylloclade $p._1$ can be traced. In Fig. 33 B the bract $br._1$ has become detached from the right-hand margin of the phylloclade—laterally, but a little to the inner side—and bears in its axil the bud $b._1$, which has produced a first leaf, $br'._1$. A little higher, only the upper part of the bract $br._1$ is visible (Fig. 33 C), while higher still (Fig. 33 E) a similar bract, $br._2$, is detached laterally, but this time a little to the outer side. As in the case of $br._1$, $br._2$ subtends a bud, $b._2$, which bears a bracteole, $br.'_2$. Figs. 34 A and B, in which transverse sections of the margins of two phylloclades are drawn at a slightly more advanced stage, show, as before, the bract *br.* and the bud *b.*, with its first leaf, *br'.*—but, in addition, they include the second leaf of the bud, *br''*. The phylloclades $p._1$, $p._2$, and $p._6$ in Figs. 33 A–G represent the normal case in which the inflorescences are confined to the margins of the phylloclades. But the same series of sections also provides examples of phylloclades of two divergent types. That marked $p._5$ in Fig. 33 G bears a bud, X, in the median line on the *abaxial* side, as well as its marginal buds. The same phylloclade, when followed to the apex, is found to bifurcate (Fig. 33 I). The phylloclade $p._7$ (Fig. 33 H), on the other hand, bears, in addition to its marginal buds, a bud, Y, in the median line on its *adaxial* surface.

Fig. 35, p. 244, represents a section through a somewhat older bud than that drawn in Fig. 33, p. 242; the vascular system is now differentiating, and



Figs. 30–34. *Semele androgyna*, (L.) Kunth. (material from Cambridge Botanic Garden). Fig. 30 A, apex of current year's shoot, Jan. 26, 1923 (× ½); *sc.*, scale-leaves; *ax.*, axis. Fig. 30 B, transverse section through bud, *b.*, in axil of one of the scale-leaves, *sc.*, from shoot similar to that drawn in Fig. 30 A; *ax.*, axis; Feb. 6, 1922 (× 23). Fig. 31 A and B, the result of further development of a bud such as *b.* in Fig. 30 B; Fig. 31 A, Feb. 19, 1923; Fig. 31 B, May 4, 1922; the scale-leaves, *sc.*, correspond in Figs. 30 A and B and 31 A and B. In Fig. 31 B the two series of leaves, *l.*, are seen to bear phylloclades, *p.*, in their axils (× ½). Fig. 32, a phylloclade, *p.*, and its axillant leaf, *l.*, in radial longitudinal section, from microtome series through apex of a shoot similar to that shown in Fig. 31 B; May 4, 1922 (× 14); the bracts borne on the phylloclade do not lie in the plane of the section. Figs. 33 A–I, transverse sections from microtome series through apex of shoot similar to that drawn in Fig. 31 B, May 4, 1922 (× 14), showing the development, in two opposite rows, of leaves, $l._1$–$l._9$, with phylloclades, $p._1$–$p._9$, in their axils. Figs. 33 B, C, E, show the development of bracts $br._1$ and $br._2$, axillary buds, b_1, and $b._2$, and bracteoles, $br'._1$ and $br'._2$ from right-hand margin of $p._1$, and Figs. 33 C and D, from left-hand margin of $p._1$. In Fig. 33 G $p._6$ is abnormal in having a bract and bud (X) in the median region, on the surface towards the axillant leaf; its apex,

it is seen that the xylem of the phylloclade bundles lies on the side towards the axillant leaf. As in the case of *Danae racemosa,* the bundle of the axillant leaf is anomalous in its structure. Figs. 38, 39, and 40, p. 244, are drawn from three young axillant leaves (June 2), each of which had one bundle only. The xylem tends to a form recalling the 'double-bundle' of cotyledons—the metaxylem consisting of two separate masses connected by the protoxylem. In Figs. 39 and 40 there is the further peculiarity that there is a development of tracheides, *cp. xy.*, on the side of the protoxylem remote from the phloem. The bundles of such leaves are also anomalous in their structure, when maturity is reached. In Fig. 36 A we have a fully developed leaf with three bundles, which lie very close to the upper surface. Each consists almost entirely of xylem—which is unusually extensive laterally—with one or two small patches of phloem. The median bundle is shown in detail in Fig. 36 B. There are three files of protoxylem elements, *px.*, and on either side of each of these there are radial files of parenchyma cells, between which the protoxylem elements are compressed. The leaf is, in all probability, of leaf-sheath nature, and these radial files of elements seem to owe their origin to that meristematic activity which is not infrequent near the ventral surfaces of leaf-sheaths. As a rule this tendency to cell-division does not involve the vascular tissue, but in this particular case the bundles lie so close to the surface that they are affected by it.

After the production of a certain number of scale-leaves with phylloclades in their axils, the growing apex of the *Semele* shoot, as a rule, aborts, its last appendages being usually a pair of scale-leaves subtending phylloclades (Fig. 46 C, p. 246). Occasionally, however, a narrow phylloclade (*p.t.* in Fig. 46 E) is produced terminally, without any axillant leaf, as the last effort of the growing point.

As a consequence of the climbing habit of *Semele*, the mature phylloclade is rather variously held, but the commonest position is a more or less horizontal one, with that surface uppermost that originally faced the axillant leaf. The inflorescence buds are produced in two series at either margin, and are supplied by a pair of conspicuous veins, which diverge from one another near the base of the phylloclade (Figs. 41 A, B, C, and 42, *v.c.*, p. 244); otherwise the venation is not pronounced, and the numerous cross-connexions between the longitudinal veins are too delicate to be shown in the sketches. But though two series of marginal buds are the rule, exceptions, such as we noticed in the case of the embryonic shoots (X in Fig. 33 G, Y in Fig. 33 H, p. 242), are so common that they cannot be ignored. Examples are repre-

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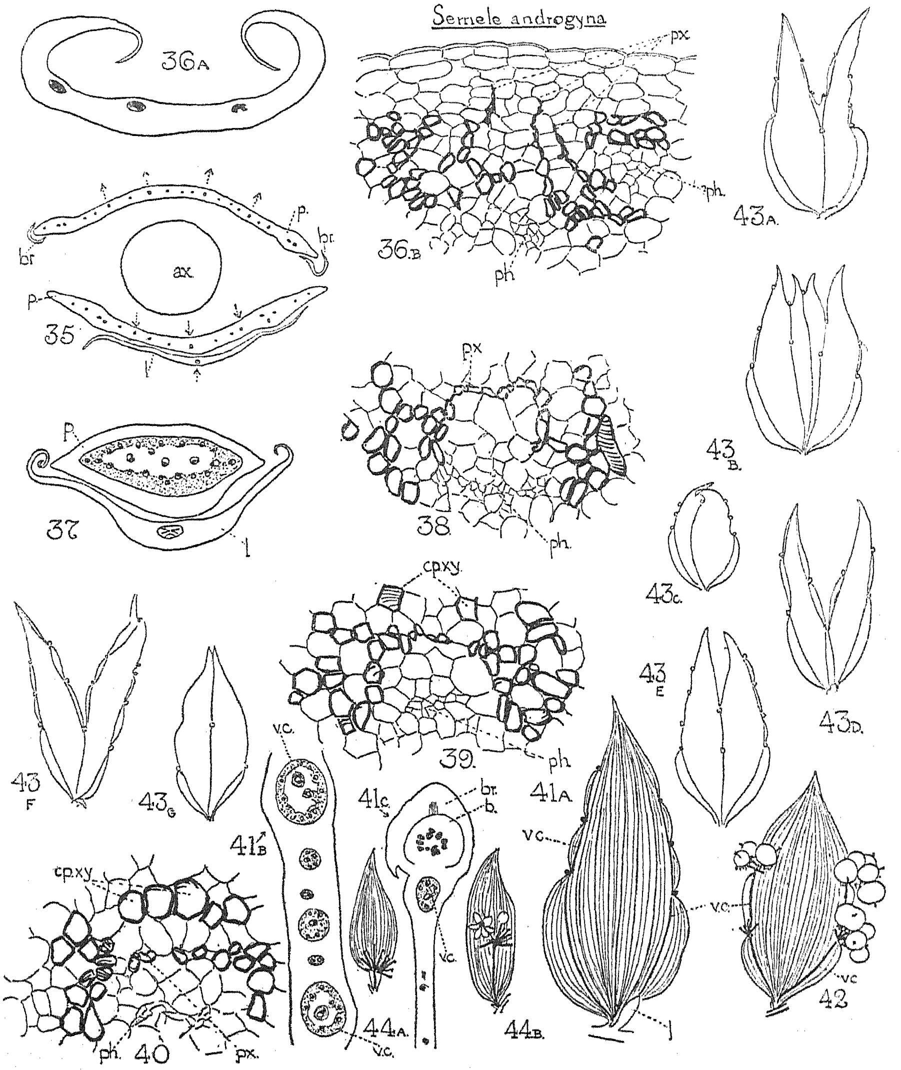
Fig. 33 I, is bifurcated. In Fig. 33 H *p*.$_7$ also has a bract and bud (Y) in its median region, but on surface remote from axillant leaf; *p*.$_7$ does not bifurcate at apex. In Figs. 33 H–I only part of section shown. Figs. 34 A and B, transverse sections of margins of two phylloclades in which axillary bud, *b.*, is more advanced than in Fig. 33, and bears two bracteoles, *br'*. and *br''*. (× 14). Fig. 34 A, from material gathered May 5, 1922. Fig. 34 B, from upper phylloclade shown in Fig. 35, p. 244, but at a lower level; June 2, 1922.

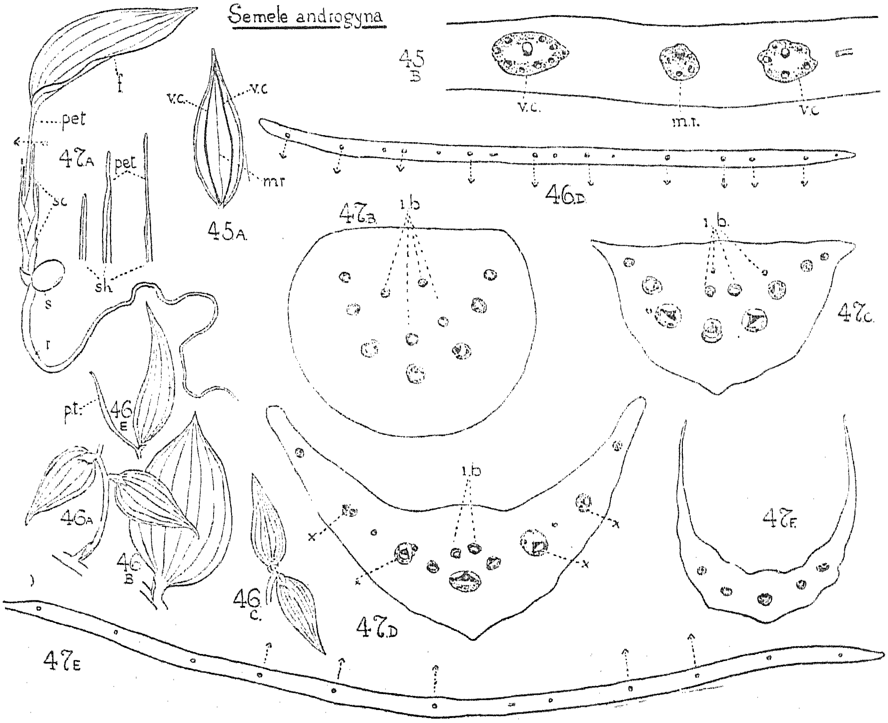


Figs. 35–44. *Semele androgyna,* (L.) Kunth. Fig. 35, transverse section through axis, *ax.*, of bud, June 2, 1922, slightly older than that shown in Fig. 33, p. 242, bearing two phylloclades, *p.*, the axillant leaf, *l.*, of the younger one being alone visible at this level; *br.*, bract; the arrows show direction of xylem in leaf and phylloclades (× 14). Fig. 36 A, transverse section (× 14) of axillant leaf, *l.*, shown in Fig. 41 A (bottom of page). Fig. 36 B, median bundle from Fig. 36 A (× 193). Fig. 37, transverse section of base of a mature phylloclade, *p.*, with axillant leaf, *l.* (× 14). Figs. 38, 39, 40, the bundle from three young axillant leaves, June 2, 1922, in which a median strand alone is developed; *px.*, protoxylem; *ph.*, phloem; *cp. xy.*, centripetal xylem (× 318). Fig. 41 A, phylloclade bearing buds, only longitudinal veins indicated; *v.c.*, main lateral veins (× rather more than ½). Fig. 41 B, section of median region of a phylloclade similar to Fig. 41 A, not far from base, to show main lateral veins, *v.c.*, which will supply inflorescence (× 14). Fig. 41 C, transverse section of margin of phylloclade drawn in Fig. 41 A, at base of one of marginal buds, *b.*, showing bract, *br.*, and vein, *v.c.*, from which vascular system of bud is derived (× 14). Fig. 42, phylloclade bearing fruits; *v.c.*, main lateral veins (× rather more than ½), Feb. 1923. Figs. 43 A–G, abnormal phylloclades, all from one plant in Camb. Bot. Garden, Jan. 1923 (× ½); buds indicated by small circles; in A, D, F, the surface towards axillant leaf is shown; in B, C, E, G, the surface away. Figs. 44 A and B, sketches from herbarium material of two phylloclades bearing flowers on the upper surface instead of at margin (× ½).

sented in Figs. 43 A–G and 44 A and B. These abnormal phylloclades are usually forked at the tip, slightly or deeply, or else fissured in a more complex fashion, thus giving opportunity for a further development of marginal buds, and additional buds may also appear on either surface, generally in the median line. In other cases (e. g. Figs. 44 A and B) the marginal buds may be altogether absent, and the inflorescence may take the *Ruscus*-like form of a single group of flowers in the median line. Abnormalities of the latter type have been, in the past, treated as a distinct species and described under the name of *Danae Gayae*, Webb et Berth. (33, p. 321, Pl. 224 B).

The anatomy of the mature phylloclades of *Semele* closely recalls that of the other two genera. Fig. 37, representing a transverse section near the base, may be compared with Fig. 4 A, p. 232, showing the same region in *Danae racemosa*, and with the corresponding sketch of *Ruscus Hypoglossum* (Fig. 21 B, p. 238). Fig. 41 B represents the median region of a fertile phylloclade, such as that in Fig. 41 A, cut near the base; the structure of the two main veins, *v.c.*, which will supply the inflorescences, is radial, as is that of some of the other veins. Fig. 41 C is from a section of the margin, cut through the base of a bract, *br.*, and its bud, which are just on the point of detachment; the bud, *b.*, has received its vascular supply from the vein *v.c.* All those bundles in the phylloclade, which are not either grouped into steles or immediately derived from the subdivision of these steles, are orientated with their xylems towards the axillant leaf. Radial veins are not peculiar to the fertile phylloclades; they are shown in Fig. 45 B, p. 246, which is a transverse section near the base of a strongly-formed sterile phylloclade, such as that drawn in Fig. 45 A. The more delicate sterile phylloclades (Fig. 46 A) may, however, show a single series of bundles only (Fig. 46 D); such phylloclades recall those of *Danae*, both in appearance and anatomy.

The seedlings of *Semele androgyna*, and sometimes the mature rhizomes, produce long-stalked foliage leaves, like those of *Danae* (7, 14, 24). By the kindness of the Director of the Royal Botanic Gardens, Kew, I have been able to examine a seedling of *Semele* which bore one such leaf, preceded by scale-leaves, and also by transitional leaves in which the leaf-base terminated in a rudimentary petiole (Fig. 47 A, p. 246). The long-stalked foliage leaf, whose anatomy is shown in Figs. 47 B–E, had in this case scarcely any basal sheath, but from the descriptions and figures in the literature it is clear that the sheath is often well developed. The petiole is noteworthy for its tendency to radial structure, indicated by the presence of bundles, *i.b.*, with their xylems directed downwards, in addition to the main arc of strands with their xylems upwards. At the base of the limb the main veins (marked with a × in Fig. 47 D) contain more than one bundle, and tend to a radial plan.



Figs. 45–47. *Semele androgyna*, (L.) Kunth. Fig. 45 A, small but strongly developed sterile phylloclade, from side towards axillant leaf, to show pair of principal veins, *v.c.*, and less prominent midrib, *m.r.* (× ½). Fig. 45 B, transverse section of median region near base of a sterile phylloclade similar to Fig. 45 A (× 23). Figs. 46 A, B, C, sterile phylloclades from feebly-developed basal shoot; A, from side towards axillant leaf; B, from side away from axillant leaf to show well-marked stalk; C, to show termination of shoot (× ½). Fig. 46 D, transverse section near apex of feebly-developed sterile phylloclade, similar to those drawn in Fig. 46 A–C (× 14). Fig. 46 E, end of a shoot from the upper part of the plant (not a feeble shoot from the base such as that used in Figs. 46 A–D) to show terminal phylloclade, *p.t.* (× ½). Fig. 47 A, seedling (× ½), Kew, Feb. 7, 1923; seed, *s.*; radicle, *r.*; scale leaves, *sc.*; first foliage leaf, *f.*, with petiole, *pet.* Some of the scale-leaves drawn separately (to the right) to show transition between those with sheath, *sh.*, alone, and those with sheath and petiole. Figs. 47 B–D, transverse sections through petiole and base of limb of leaf *f.* in Fig. 47 A (× 23). Fig. 47 B, low in petiole. Fig. 47 C, at level of arrow in petiole. Fig. 47 D, at base of limb. The bundles marked *i.b.* have their xylems directed downwards; the veins in Fig. 47 D marked with a × contain more than one bundle and are more or less radial. Fig. 47 E, transverse section near apex of same leaf (× 14). Fig. 47 F, transverse section of sheath region of one of the transitional scale-leaves in Fig. 47 A, slightly reconstructed at margins, as section imperfect (× 23).

3. THE MORPHOLOGICAL INTERPRETATION OF THE PHYLLOCLADES OF THE RUSCEAE.

(i) *Historical.*

Three hundred years before the beginning of the Christian era, Theophrastus (28), in describing two species of *Ruscus*, wrote as follows: 'Both have their fruit on the midrib of the leaf.'[1] This represents the earliest expression of the view that treats the phylloclades as foliar—a view which remained undisputed, so far as I can learn, until the nineteenth century. It was about a hundred years ago—at the period when morphological ideas were beginning to crystallize—that the opinions of botanists upon the structure of the Rusceae suffered a revulsion. Turpin (30) in 1820 first put forward the theory that the phylloclade was 'un . . . rameau aplati, foliacé, florifère', and this view of the purely caulome nature of the organ has since been very generally adopted. It seems to have found one or more supporters in every decade of the century that has passed since it was first suggested; it would be impossible to enumerate them all, but we may recall that it was upheld by de Candolle in 1827 (9), Saint-Hilaire in 1840 (25), Nees von Esenbeck in 1843 (22), Kunth in 1850 (20), Schacht in 1853 (26), Clos in 1861 (12), Askenasy in 1872 (4), Cauvet in 1877 (10), Dickson in 1886 (14), Čelakovský in 1893 (11), Dutailly in 1896 (15), Reinke in 1898 (24), Bernatsky in 1905 (6), Szafer in 1910 (27), and Goebel at various dates, including 1922 (18). It has also found general acceptance in systematic works and text-books, e. g. Engler (17) and Baillon (5).

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[1] ἀμφότεραι γὰρ τὸν καρπὸν ἔχουσιν ἐκ τῆς ῥάχεως τοῦ φύλλου. Enquiry into Plants, III. xvii. 4.

But the view which treats the phylloclade as exclusively axial has not passed altogether unchallenged. Velenovský (31, 32), supported more recently by Daněk (13), has maintained a different opinion, namely, that in the flowering phylloclade of *Ruscus* the proximal part, up to the level of the bud, is a winged inflorescence axis, whereas the distal part is purely foliar. A slightly different interpretation, which seems, however, to be related to that of Velenovský, has been indicated by Buscalioni in a recent preliminary note (8).

There is yet a third theory, which has received little attention, and which does not seem to have been accepted by any recent writer. This view, which was first suggested by Koch (19) in 1837, and which was subsequently revived and developed by Duval-Jouve (16) and van Tieghem (29), is that, in the case, for instance, of the fertile phylloclade of *Ruscus*, an inflorescence axis bearing a prophyll (bracteole) arises in the axil of a scale-leaf; the prophyll (phylloclade) develops on an unusual scale, and the inflorescence axis is adnate to it, up to the level of the second leaf (the bract below the inflorescence).

(ii) *Critical.*

The three theories enumerated in the preceding paragraphs appear to have exhausted the alternatives—at least as regards the main lines of the interpretation—so we have to decide with which of them the balance of probability lies.

(a) *The 'Axial' or 'Caulome' Theory.*

The first view, which we may call for simplicity the caulome theory, is based upon two axis-like characters presented by the phylloclade—firstly, its occurrence in the axil of a leaf, and, secondly, the fact that it produces one or more bracts and buds. It seems, at first glance, not unnatural to take these two points as indicative of caulome nature, but, nevertheless, if we treat the phylloclade as a flattened axis, we find ourselves led into certain difficulties. The sterile phylloclades, for instance, must, if they are caulomes, be interpreted as naked axes, bearing neither leaves nor leaf rudiments; it may, however, be claimed that this condition, though it is extremely rare, finds a precedent in certain thorns and tendrillar shoots. Again, on the caulome theory, while the fertile phylloclades of *Ruscus* must be explained as formed of two internodes, the sterile phylloclade cannot be treated as consisting of more than one; such a difference in the interpretation of these two organs, which are often indistinguishable when viewed from the abaxial side, seems to me to be highly artificial. But a more serious objection to the caulome theory is that the anatomy of the phylloclades is by no means what experience leads us to look for in a flattened stem. Such stems—ruling out those winged by decurrent leaf-bases, which cannot be brought into the present comparison—are rare, and the best example seems to be *Muehlenbeckia platyclados*, Meissn. (4), a member of the Polygonaceae, in which the ribbon-like stem is divided into segments by the lines of insertion of the reduced leaves. A transverse section across one of these internodes shows that the bundles are arranged in an extremely flattened ellipse; there is no essential change from the structure of a normal stem, except that the bundle ring is flattened out. Reference to the figures and descriptions in the earlier part of this paper will show that there is no phylloclade among the Rusceae in which the structure is in any way comparable with that of *Muehlenbeckia platyclados*. With the exception of the strands forming the principal veins, which may be radially grouped, the phylloclade bundles, in all five species belonging to the Rusceae, are orientated in the same sense, with the xylem towards one surface of the phylloclade and the phloem towards the other; I know of no stem in which such a vascular scheme prevails, and the plan of the skeletal system thus seems to me to preclude the axial interpretation.

(b) *The 'Winged Axis and Bract' Theory.*

The second interpretation, according to which the fertile phylloclades of the Rusceae are axial in the proximal and foliar in the distal region, is most definitely expressed in the later of Velenovský's pronouncements (32) and in Daněk's more elaborate development of the same ideas (13). On this view the sterile phylloclade of *Ruscus* is the terminal leaf of a brachyblast, which aborts and bears no other appendage, while the fertile phylloclade consists of a winged inflorescence axis, passing over into a bract in its distal region. The flowering phylloclade of *Semele* is regarded as equivalent to as many fertile *Ruscus* phylloclades as there are inflorescences, congenitally fused. I do not propose to spend time over this theory, because it seems to me that the elements of truth which it contains are to be found in those points which it has in common with the third theory, which we shall next consider; it has, on the other hand, certain disabilities peculiar to itself. To suppose that, in the fertile phylloclade of *Ruscus*, the region up to the level of the flower-bud is axial, while the region above this level is foliar, seems to me to be contrary to common sense. And I find it equally difficult to imagine how the lower half of the fertile phylloclade can be visualized as axial, when the corresponding, and closely similar, region in the sterile phylloclade is classed as foliar.

(c) *The 'Prophyll' Theory.*

We now come to the third and last theory, which we owe to Koch (19), Duval-Jouve (16), and van Tieghem (29). Although this view appears to have found no supporter for the last forty years, the present study of the Rusceae has led me to believe that it represents the closest approximation to the truth which has yet been reached; I think, however, that it requires some modification. According to the view in question, the phylloclade is the first leaf (prophyll or bracteole) borne on a branch of limited growth, which may either bear no further appendages (all phylloclades of *Danae*; sterile phylloclades of *Ruscus* and *Semele*) or may pursue its development, while adnate to the prophyll, and bear bracts and buds (fertile phylloclades of *Ruscus* and *Semele*). I think it will make for clearness to treat the different elements of this theory separately, so I will first consider the evidence for the foliar interpretation of the phylloclade; secondly, the evidence for its prophyllar nature; and, finally, the fate of the axis which bears it, and the question of its adnation to its own prophyll.

In favour of the view that the phylloclades of the Rusceae are of foliar nature, it may be recalled that, in their form—especially in the relation of the short, stalk-like basal region to the expanded limb—and in their venation, they closely resemble the leaves of various related Liliiflorae (13). This resemblance is illustrated in Figs. 48 A–C, p. 250, for the case of *Smilacina* and *Polygonatum* (Polygonatae) and *Luzuriaga* (Luzuriageae), members of the two tribes of Liliaceae which Bentham and Hooker treat as following most closely upon the Asparageae (*Ruscus*, *Semele*, *Danae*, *Asparagus*). The leaf of *Luzuriaga* resembles the phylloclades of the Rusceae, not only in its form, but also in its tendency to basal torsion—a tendency even more clearly displayed in the similar leaf of the Amaryllid *Bomarea* (Fig. 48 D), which is remarkably like some of the phylloclades of *Danae* and *Semele*, with their narrow twisted bases.

Another argument, which lends support to the foliar theory, is that *Ruscus*, and *Semele* also, is capable of producing phylloclades directly from the axis, without any axillant leaf. Almost every shoot of the three species of *Ruscus* terminates, as has long been known, in a phylloclade borne in this way (26, 10, 15). This fact is indicated in many of the sketches here reproduced; the terminal phylloclade, marked *p.t.*, is shown for *Ruscus aculeatus* in Figs. 8 D, 9 C, and 11 C, p. 236; for *R. Hypoglossum* in Figs. 20 B, C, D, p. 238; for *R. Hypophyllum* in Figs. 25 A and B, p. 240. So far as I know, nothing of the kind has hitherto been recorded for *Semele androgyna*,[1] but I have recently noticed that, although most of the shoots end in a pair of scale-leaves with phylloclades in their axils (Fig. 46 C, p. 246), a certain number bear at the tip only a single axillant leaf with its phylloclade, the latter forming a pair with a much-reduced terminal phylloclade (Fig. 46 E, p. 246). It may be thought, at first glance, that the fact that phylloclades can be terminal to an axis militates against the foliar view of their nature. But Queva's work on *Uvularia grandiflora*, Sm. (Liliaceae), has shown that a normal leaf can be practically terminal (23). I have tested Queva's conclusion by cutting microtome series through the tip of a shoot of a species of *Uvularia*, and in one case I found the stem apex reduced to a minute non-vascular cone, completely adherent to the base of the last leaf. Such a condition of the growing point may seem wholly anomalous to those who habitually think of buds from the standpoint of the Dicotyledon, but it will not, I think, seem at all unnatural to those who are accustomed to

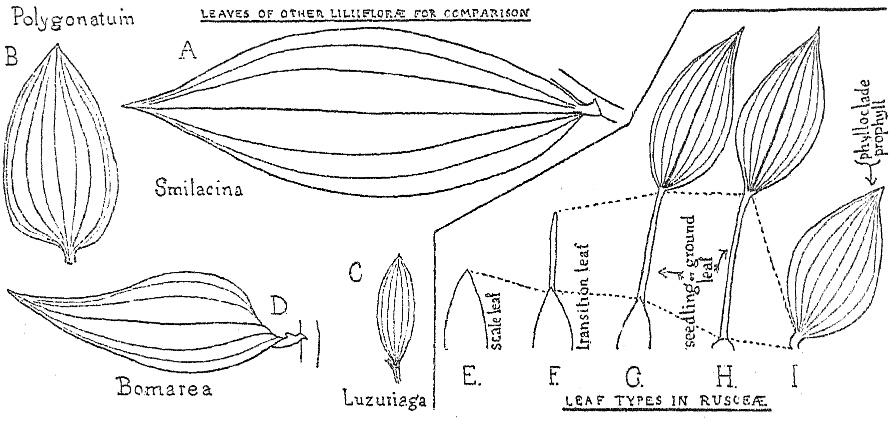

Fig. 48 A–D, leaves of other Liliiflorae for comparison. A, *Smilacina racemosa*, Desf. (× ½). B, *Polygonatum officinale*, All. (× ½). C, *Luzuriaga* sp. (× 1). D, *Bomarea* (garden hybrid) (× ½). Figs. 48 E–I, diagrams of different leaf types met with in the Rusceae, to illustrate interpretation of phylloclade put forward in the present paper. E, scale leaf, consisting of sheathing leaf-base only; F, transitional leaf (e.g. seedling of *Semele*); G, foliage leaf with well-developed sheath, petiole, and limb (such leaves are borne by seedling and sometimes by rhizome of *Danae* and *Semele*); H, similar leaf with reduced sheath (e.g. the leaf figured here for seedling of *Semele*, Fig. 47 A, p. 246); I, phylloclade (e.g. those of *Danae*, and sterile phylloclade of *Semele*, Fig. 46 B, p. 246). The dotted lines show the limit of sheath, stalk, and limb in the different leaf categories.

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[1] Čelakovský (11) states that terminal phylloclades are peculiar to *Ruscus* and never occur in *Semele* and *Danae*.

looking at embryonic Monocotyledonous shoots, in which the leaves often play a much more conspicuous part than the axis which bears them.

But the question of the terminal phylloclade—though it is an important one—has a less direct bearing on our present problem than another point to which we must now turn—the comparison between the phylloclades of *Danae* and *Semele* and the limbs of the ground leaves produced by the same genera. It may be recalled that we have in *Pinus* a precedent for the production of juvenile leaves directly from an axis, followed, at maturity, by leaves borne on lateral short shoots. I have attempted to show in Figs. 48 E–I that, if we regard the phylloclades as leaves, we can trace a nicely graduated series, from scale leaves of purely leaf-base nature (Fig. 48 E), through scale-leaves in which the base is reduced and there is a petiolar apex[1] (F), to foliage leaves in which a long petiole terminates in a limb and in which the leaf-sheath may be conspicuous (G) or poorly developed (H), and finally to phylloclades (I), in which the sheath is absent and there is merely an extremely reduced petiole and a well-developed limb. We will return on p. 255 to the evidence for the petiolar nature of the base of the phylloclade. As I have attempted to explain in previous papers (1, 3, &c.), I look upon the blade-like limb of Monocotyledonous leaves as an expansion of the distal region of the petiole; and since I regard the phylloclade as a leaf, I should naturally apply the same interpretation to its limb. But, so far as the present paper is concerned, this is a side issue, and the theory of the phylloclade, here adopted, stands or falls independently of the phyllode theory of the leaf.

The hypothesis which we are now considering does not stop at treating the phylloclades as leaves; it goes farther, and relegates them to a definite category—that of *prophylls* or *bracteoles*. The idea of regarding these large and well-developed organs as prophylls may perhaps demand some readjustment of one's general notions about the relation of the prophyll to the rest of the shoot. As a rule the prophylls of the vegetative shoots, and their equivalents, the bracteoles of the reproductive shoots, are small and unimportant objects, both in comparison with the lateral axis which bears them, and with the leaf which subtends this axis. But, as Duval-Jouve (16) pointed out, the relatively great size of the prophyll in the Rusceae is probably to be correlated with the minimal character of the lateral axis that produces it, and with the reduction to a mere caducous scale of the leaf subtending this axis. The prophyll has, as it were, assumed the predominance among this group of organs, and, in the course of its disproportionate development, it has completely mastered the growing apex of the axis which bore it.

It is characteristic of Monocotyledons that the prophyll often shows a precocious development; it is possible, indeed, to find extremely young bud rudiments, in which little besides the prophyll is recognizable. Figs. 50 A–C, p. 257, represent three transverse sections from a series through an axillary bud of *Rhipogonum album*, R. Br. (Liliaceae); this bud was only about half a millimetre in width, and, as the sketch shows, at this stage the prophyll, *pr.*, was very much more conspicuous than the growing apex, *ap.*, which represented the remainder of the bud. If the prophyll retained through life the ascendancy over the other elements of the shoot, which is not infrequent in these early stages, we should have something comparable with the conditions prevailing in the Rusceae. And it may be suggested that, in their exaggerated prophyllar development, the Lemnaceae offer a case to some extent analogous with the Rusceae, for in the Duckweeds the whole vegetative shoot system consists of reduced axes, each bearing a prophyllar leaf (2).

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[1] Transitional leaves of a somewhat different type have been described for *Danae* (27, p. 263, and Figs. 26 and 27, p. 269).

I should like also to suggest that there are two characters, visible on naked-eye examination, which seem to favour the identification of the phylloclades of *Semele* and the prophylls of other Monocotyledons. One of these is the lack of a predominating midrib (Figs. 46 A and B, p. 246), a character which is also shared by *Danae* (Fig. 3 A, p. 232); while the other is the major importance assumed by the two main lateral veins (*v.c.*, Figs. 41 A and 42, p. 244, and Fig. 45 A, p. 246). A bi-keeled form with two principal veins, neither of which is in the median position, is a common character of Monocotyledonous prophylls, and the condition in *Semele* can be paralleled, in detail, in the prophylls of another member of the Liliaceae, *Ornithogalum caudatum*, Ait. (21), in which the midrib, though present, is smaller than the two main laterals. An entirely different explanation of the presence of these two prominent lateral veins in *Semele* has been given by van Tieghem, who regards them as due to the adnation to the prophyll of a double cyme consisting of a pair of sympodia. To this view there seem to me to be two serious objections: firstly, that these two conspicuous lateral veins may also be found in a sterile phylloclade (e.g. Figs. 45 A and B, p. 246); and, secondly, that there is no good evidence that the set of flower clusters borne on the phylloclade of *Semele* form, *in their entirety*, a cymose inflorescence. There is no terminal flower at the point from which the two veins diverge, and the marginal flower-buds are not themselves terminal, but arise in the axils of bracts (e.g. Figs. 33 and 34, p. 242). And, moreover, I do not think that we have any reason to assume that the character of the inflorescence in *Semele* has determined the position of the main veins in the phylloclades; it appears to me much more probable that, throughout the Rusceae, the flower-buds have followed the principal veins of the prophyll, simply because, without a supply of food and water, they could not develop. But the question arises, why is it that in *Semele* the two main lateral veins predominate, while in *Ruscus* the median vein retains its supremacy, and the flowers, correspondingly, occur in the median line? It seems to me possible that the difference between the phylloclades of the two genera is to be related to the conditions in the bud. There is little doubt that, in Monocotyledons in general, the midrib-less, bi-keeled form of the prophyll is due to compression of its median region during development; the prophyll is, as it were, squeezed between the axillant leaf and lateral axis on one side, and the main axis on the other. Now the two-ranked arrangement of the leaves found in *Semele* (Fig. 33, p. 242), which is a common form of phyllotaxis throughout Monocotyledons, affords just those conditions of close packing under which the main laterals of the prophyll are likely to develop more conspicuously than the median strand. But in *Ruscus* the spiral phyllotaxis (Figs. 11 A and B, p. 236) gives greater freedom, and the median strand is thus able to hold its own.

In comparing the phylloclade to a single prophyll facing the axillant leaf, which is the rule among Monocotyledons, we may be met by the objection that the branches of the Rusceae are apt to have their first leaves laterally placed, so that they do not conform to the Monocotyledonous type, but almost recall the paired prophylls of a Dicotyledon (e.g. *Danae racemosa*, *l.* and *l.*, Fig. 1 I, p. 231; *Semele androgyna*, $l._1$ and $l._2$, Fig. 30 B, p. 242). But I do not think that this objection carries much weight, since in the buds borne on the phylloclade of *Ruscus aculeatus* we find that the two first leaves may either be arranged transversely (*br.′* and *br.″*, Fig. 19 E, p. 236) or else so obliquely that the first almost faces the axillant bract (*br.′*, Fig. 16, p. 236); an oblique placing of *br.′* in relation to *br.* is also characteristic of the inflorescence buds of *Semele androgyna* (Figs. 34 A and B, p. 242). It is conceivable that the bifid phylloclades, which frequently occur in *Semele* (Figs. 43 A–G, p. 244), may represent cases in which the phylloclade is equivalent to two transversely placed prophylls, congenitally fused to form a single organ facing the axillant leaf.

There seems, then, to be little difficulty, on general grounds, in regarding the phylloclades (except those terminal to the long shoots) as prophylls, and when we turn to the internal structure we find that this view is reinforced by strong and definite anatomical evidence. A feature common to the phylloclades—sterile and fertile—of all the five species of the Rusceae is that, in those veins which consist of a single bundle, *the xylem is directed towards the axillant leaf.* This peculiarity was first recorded by van Tieghem (29), who was also the first to appreciate its significance; it is illustrated for *Danae racemosa* in Figs. 2 B and C, 4 B, 5 and 6 A, p. 232; *Ruscus aculeatus*, Figs. 10 and 19 D–F, p. 236; *R. Hypoglossum*, Figs. 21 D and K, 23 A and B, p. 238; *R. Hypophyllum*, Figs. 26 B and 28, p. 240;

Semele androgyna, Fig. 35, p. 244, Fig. 46 D, p. 246. On the caulome theory, this peculiar and consistent orientation of the bundles is inexplicable, but it is, on the other hand, precisely what one would expect if the phylloclade is the prophyll of a branch subtended by the axillant leaf, and, like the majority of Monocotyledonous prophylls, 'adossiert' in relation to the main axis.

Turning from the consideration of the prophyll itself to that of the hypothetical axis which bears it, we find ourselves involved in some obscurity, because Duval-Jouve (16) and van Tieghem (29) gave an account of the anatomy which, though it perfectly supported the idea of adnation—as they understood this term—does not, unfortunately, represent the facts. One would gather from their descriptions that, while in *Ruscus* the sterile phylloclade and the distal region of the fertile phylloclade have an obviously foliar type of anatomy, with a single series of bundles lying in one plane, the fertile phylloclade has, in the proximal region, a median cylinder of bundles, suggesting stem anatomy, which passes into the inflorescence axis at its point of detachment. But this description, though it accords diagrammatically with these authors' conception of the adnation theory, is altogether too simple. I have found that in the phylloclades of *Ruscus* and *Semele* the principal bundles are often radial in structure, *whether they bear any relation to an inflorescence or not.* This is demonstrated by many of the sections illustrated in the earlier part of this paper. We may take the case of *Ruscus Hypophyllum* as an example, since it was that on which van Tieghem based his description. From his account it would appear that there is only one vascular cylinder, which passes from the base of the phylloclade to the inflorescence which it supplies. I have found, however, that a section cut near the base of a fertile phylloclade may show no less than five radial bundle groups, occupying the principal veins, only one of which is destined to be associated with the inflorescence (Fig. 26 B, p. 240). Figs. 27 C, D, E, again, show that radial structure may be retained in more than one vein in the distal region of the phylloclade above the inflorescence. Fig. 28 also reveals two radial veins in a section cut not far from the base of a sterile phylloclade. *Ruscus Hypoglossum* gives exactly comparable results (see Figs. 21, 22, 23, p. 238);[1] while in *R. aculeatus*—though its vascular system is reduced as compared with the other two species—we may find a vascular cylinder in the midrib even of the sterile phylloclade, if it is a well-grown example (Fig. 14, p. 236). In the fertile phylloclade of *Semele androgyna*, again, it is not only the two principal veins supplying the inflorescences which are radial, but also others lying between them (Fig. 41 B, p. 244), and vascular cylinders may also be found in the sterile phylloclade (Fig. 45 B, p. 246).

But, despite the errors in Duval-Jouve's and van Tieghem's descriptions, I am inclined to believe that they were essentially right in claiming the fertile phylloclade of the Rusceae as representing a case of adnation between an inflorescence axis and a prophyll. But I think they should have gone farther, and instead of raising an artificial barrier between the sterile and the fertile phylloclades, by regarding the former as terminal to an abortive axis, while restricting the idea of adnation to the latter, they should have treated all the phylloclades as alike formed by adnation between a lateral axis and its prophyll—the only difference between the two types being that that which is described as 'sterile' bears no other appendages after the prophyll, while the 'fertile' or 'floriferous' phylloclade, on the other hand, produces one or more bracts subtending buds. I do not think it is possible, in either case, to decide precisely where the adnate axis ends; there is obviously no criterion for this determination in the case of the sterile phylloclade, and even in the fertile phylloclade we cannot come to any final conclusion, because the flower-buds all arise laterally in the axils of bracts, so that there is no necessity to treat the inflorescence—as van Tieghem does—as marking the termination of the axis. In this and other points of interpretation, I think that Duval-Jouve and van Tieghem were misled by their failure to realize that the union which the adnation represents is a fusion of an excessively intimate nature, in which one partner—the prophyll—has gained such complete ascendancy that it is useless to look for the structural boundaries of the adnate axis; the prophyll has, as it were, absorbed the axis without making any concessions to it, and has retained its own foliar type of anatomy unaltered.

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[1] Since this paper was in print, I have learned that the numerous vascular cylinders in the phylloclade of *R. Hypoglossum* were described by P. Falkenberg, *Vergleichende Untersuchungen*, 1876.

It has been usual for those who have dealt with the morphology of the phylloclade—whether they have taken the axial or the foliar view—to treat the extreme base of this organ as axial, and to point to its 'radial' anatomy as a proof of their contention. The structure of the region in question is demonstrated for *Danae racemosa* in Fig. 4 A, p. 232; *Ruscus aculeatus*, Fig. 19 A, p. 236; *R. Hypoglossum*, Fig. 21 B, p. 238; *Semele androgyna*, Fig. 37, p. 244. (In all these drawings the morphologically upper surface of the phylloclade is turned towards the lower margin of the page.) It is true that the type of anatomy revealed by these sections may—in a broad and sketchy sense—be called 'radial'; it is not, however, the genuinely radial symmetry of an axis. It seems to me, on the contrary, to indicate that the base of the phylloclade is petiolar, rather than axial, for it shows just that compromise between dorsiventrality and radial symmetry which characterizes many leaf-stalks. This petiolar interpretation is indicated in the diagrammatic sketch, Fig. 48 I, p. 250; the strongest argument in its favour seems to me to lie in the fact that the petioles of the long-stalked foliage leaves of *Semele androgyna* have themselves a decided tendency to a 'radial' type of structure; this is shown in Figs. 47 B and C, p. 246, and still more distinctly in the petiole of a better developed leaf of the same species figured by Dickson (14. Pl. IX, Fig. 3). There is a hint of the same tendency in the occurrence of inverted bundles in the petiole of the ground leaf of *Danae* (Fig. 1 B and C, p. 231; see also 27, Fig. 29, p. 269).

Not only the structure of the extreme base of the phylloclade, but also the occurrence of those 'radial' bundle-groups in its limb, to which we have referred on p. 254, have been claimed as indications of a structure at least partially axial. But the presence of these vascular groups, which may, indeed, be called distinct steles, is not necessarily an axial rather than a foliar character. In foliage leaves, even within the family Liliaceae, this type of structure can be paralleled. *Arnocrinum Drummondii*, Endl., for instance, has a leaf in which I have seen as many as seven distinct steles; the sections shown in 3, Fig. 16 B, p. 454, may be compared with that of the phylloclade of *Ruscus Hypophyllum* given here in Fig. 26 B, p. 240. And, moreover, it is not necessary to leave the Rusceae in order to find radial veins in an expanded foliar member. Fig. 47 D, p. 246, shows structure of this type in the basal part of the limb of the foliage leaf borne by a seedling of *Semele androgyna*; the compound veins are each marked with a ×.

Duval-Jouve compared the fertile phylloclade of *Ruscus* to the bracteole of *Tilia*,[1] with its adnate inflorescence axis. This comparison is, I think, perfectly valid, but unfortunately he vitiated it by describing the bracteole of *Tilia* as showing, above the inflorescence, the organization of a leaf, pure and simple, and, below the inflorescence, the organization of an axis fused with the leaf. This account is entirely wrong, and was indeed immediately corrected in a note by Cornu which follows Duval-Jouve's paper. According to current morphological ideas, we have in *Tilia* a foliage leaf (*l.*, Fig. 49 A) in whose axil an inflorescence, *i*, arises. This inflorescence has, as is usual in Dicotyledons, two prophylls or bracteoles; one (*p.*) is highly developed, and the inflorescence axis is, for some distance, fused with its upper surface. The second of these paired bracteoles is reduced and scale-like, and bears a bud, *b.*, in its axil. The sections drawn in Figs. 49 B–D show that the vascular skeleton gives no indication of the fusion of bracteole and inflorescence axis. In the midrib of the bracteole, below the inflorescence, there is a ring of bundles, but there is no reason to interpret this as anything but a purely foliar arrangement, since the midrib of the normal foliage leaf also has a bundle ring. *Tilia*, therefore, offers us an analogy for the Rusceae in having its inflorescence axis so completely merged in the prophyll that, until just below the point at which the two organs separate, the anatomy remains completely foliar.

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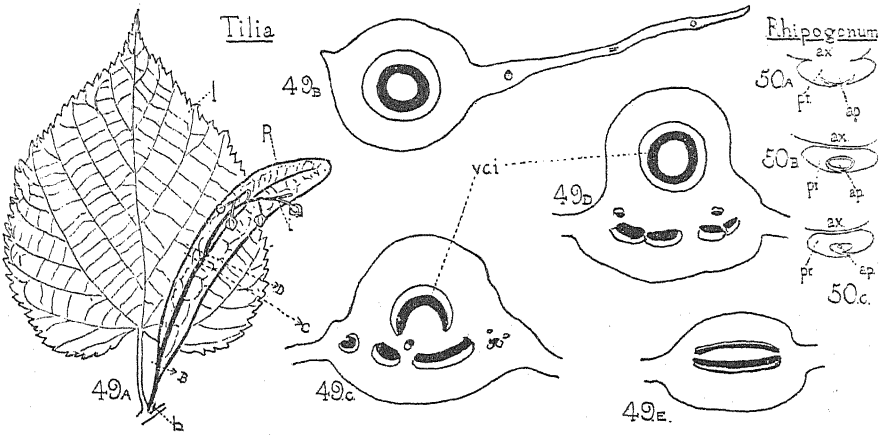
[1] This comparison was suggested in 1820 by Turpin (30).

I think that if we accept the view that, in the Rusceae, the adnation between prophyll and axis is so intimate that it is no longer revealed even by the anatomy, which remains in all respects that of a leaf, we shall find it less impossible to account for certain anomalies in the relation of the inflorescence to the phylloclade, to which attention was drawn by van Tieghem, and which otherwise stand in the way of the acceptance of the prophyllar theory. If the phylloclade is the prophyll (or bracteole) of the inflorescence axis, we should naturally expect to find the bract and the flower-buds given off on the side of the prophyll which lies towards the axillant leaf, and away from the main axis. This expectation is realized in most, but not all, of the phylloclades of *Ruscus Hypophyllum* and in some of those of *R. Hypoglossum*; but sometimes in *R. Hypophyllum*, generally in *R. Hypoglossum*, and practically always in *R. aculeatus*, the bract and bud are found on the side of the phylloclade towards the main axis. This characteristic and peculiar position is shown, for *R. aculeatus*, in Figs. 12 D, 13 A and B, and 15, p. 236. In *Semele androgyna*, although the inflorescence buds are, normally, marginal, they are sometimes median like those of *Ruscus*, and may then appear on either face (e.g. X in Fig. 33 G, and Y in Fig. 33 H, p. 242, and Figs. 43 and 44, p. 244). *Ruscus* may also, as an abnormality, show an approach to *Semele* in the marginal placing of the buds (e.g. Fig. 24, p. 238, and Fig. 29, p. 240). If, in the adnation



Figs. 49 and 50. Cases for comparison. Figs. 49 A–E, *Tilia* sp. Fig. 49 A, leaf, *l.*, with axillary inflorescence, *i.*, partially fused with its bracteole, *p.*; *b.*, bud in axil of second bracteole (×½). Figs. 49 B–E, series of transverse sections (×23) showing diagrammatically the plan of the vascular structure of midrib of bracteole; xylem, black; phloem, white. Fig. 49 B, at level of arrow B in Fig. 49 A. Fig. 49 C, at level of arrow C. Fig. 49 D, at level corresponding to arrow D, but from another bracteole; the vascular tissue marked *v.c.i.* in C and D is destined for the inflorescence. Fig. 49 E, at a level between D and apex of bracteole, but from another bracteole. Fig. 50 A–C, *Rhipogonum album*, R.Br., section from series from below upwards through very young axillary bud. Axillant leaf would lie towards lower margin of page; *ax.*, axis; *pr.*, prophyll; *ap.*, growing apex of bud (×23).

of the inflorescence axis and prophyll, the axis retained its individuality to the extent postulated by Duval-Jouve and van Tieghem, the way in which the bracts and buds emerge indifferently from either surface of the phylloclade would be incomprehensible. But when we have once realized the degree to which the prophyll has made itself master of the situation, and the completeness with which the axis has lost its anatomical independence, the vagaries in the situation of the buds become slightly less surprising, though they still remain an intriguing subject, on which we want further light. It would be of great interest to know whether corresponding variations in the normal relation of bracteole and inflorescence ever occur in *Tilia*; so far as I know, no anomalous cases of this type have ever been recorded in the Lime, but it is possible that something of the kind might be discovered if a search through a large mass of material were undertaken.

SUMMARY.

The present paper records the results of a re-examination of the vegetative structure of *Danae*, *Ruscus*, and *Semele*, special attention being paid to the anatomy, and, more particularly, to the relations of the members of the shoot, as revealed by serial sections of the developing buds.

The various morphological interpretations that have been suggested for the phylloclades of the Rusceae are then reviewed, and it is concluded that the facts of the case are best met by a theory developed from that of Theophrastus (28), Koch (19), Duval-Jouve (16), and van Tieghem (29). According to the interpretation here proposed, the phylloclades of the Rusceae, whether sterile or fertile, are all foliar (see Figs. 48 E–I, p. 250). The great majority (excluding merely those which terminate the long shoots of *Ruscus* and sometimes of *Semele*) represent prophylls (bracteoles), each borne on a lateral axis subtended by a scale-leaf; the lateral axis is, throughout its length, congenitally fused with the prophyll, to which it is so completely subordinated as to lose all vestige even of anatomical independence, with the result that the phylloclade is foliar in structure from the extreme base upwards. The adnate axis may either bear no second appendage after the prophyll (all phylloclades of *Danae* and sterile phylloclades of *Ruscus* and *Semele*), or it may be responsible for one or more bracts with buds in their axils (fertile phylloclades of *Ruscus* and *Semele*).

BALFOUR LABORATORY, CAMBRIDGE.

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The Effect of General Anaesthetics on the Respiration of Cereals.

I. Carbon Dioxide Production.

BY

EDITH PHILIP SMITH.

With nine Figures in the Text.

ANAESTHESIA may be defined as a reversible diminution of irritability. It is associated with changes in the oxidative processes (respiration) of the cell, and it may be possible to arrive at an answer to the question of the fundamental nature of anaesthesia by studying the effect upon respiration of substances known to possess anaesthetic properties. Plant tissues are particularly suitable for this purpose, since there are no complications introduced by muscular action.

For practical clinical purposes anaesthetics may be divided into general (acting upon the central nervous system) and local (acting upon a limited portion of nerve). Of the first class, ether, chloroform, and alcohol are typical; of the second, the cocaine derivatives. A comparative study has been made of ether, chloroform, and ethyl alcohol in respect of their action on the carbon dioxide output of wheat, rice, and oats. The oxygen intake, permeability to carbon dioxide, and cytological changes will be dealt with subsequently.

MATERIAL.

The material used was wheat, strain Garton's 'Little Joss', crop of 1922, from the East of Scotland Agricultural College: oats, pure-line 'Sandy', from the Scottish Society for Plant Breeding, Corstorphine: rice, a pure-line cultivated strain from Nagpur. The dry seeds were treated with hydrogen peroxide for ten minutes, to subdue the growth of moulds, washed in running water ten minutes, and germinated in sterile Petri dishes in the dark, at a temperature of about 65° F. The seeds were just covered with boiled tap-water to start with, and usually no more water was needed. Under

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these conditions the rice seedlings were ready for use in 3–4 days, the wheat in 4–5 days, and the oats in 5–6 days. The plants were taken when the roots were one-half to three-quarters of an inch long, with abundant root-hairs. The plumule was just showing, but without chlorophyll. The average number of seeds used in an experiment was twenty. Of the three, the wheat was the most satisfactory, both as regards the uniformity of germination and freedom from fungal attack. The oats were particularly susceptible to moulds, so that after some preliminary trials they were discarded, as there appeared to be no qualitative difference in their reaction to the anaesthetics used.

METHOD.

The carbon dioxide output was measured by the direct indicator method of Haas (1). The seedlings were put into a test-tube of resistance glass with a standard volume of water containing phenol red as indicator (three drops of 0·01 per cent. aqueous solution to 10 c.c. water), and the tube closed: the seedlings are thus *immersed* in a known volume of water + indicator, and the carbon dioxide given off by them in respiration dissolves in the water. The original pH value of the solution was determined by comparison with Palitzsch's borax buffers (4), contained in tubes of the same size and having the same concentration of indicator. The time taken for the carbon dioxide produced by the respiring material to change the pH of the water from one known value to another more acid is noted, the same pH interval being used as standard in all experiments. In this case the pH values used were 7·36 and 7·09. When the more acid value is reached, the water is poured off, the seedlings rinsed three times with 10 c.c. of water (of the same temperature and pH value as before), and allowed to drain for five seconds: a fresh supply of water and indicator is then added, and another reading made. This is repeated until a practically constant value is reached. The mean of several (five to ten) observations is taken as the standard. Provided that the temperature is controlled, the readings should not vary more than 5 per cent. from the mean, and with favourable material the probable error of the mean can be quite easily reduced to 2 per cent. of the mean.[1] In these experiments, the normal rate of respiration is taken as the reciprocal of the average time required to change the pH of 10 c.c. of tap-water from 7·36 to 7·09, and is expressed for purposes of comparison as 100. Deviations from the normal are expressed as percentages of the normal. Once a constant rate was attained, it would remain for six to eight hours: as the average experiment lasted two to four hours, it may be taken that variations during that period were not due to manipulation.

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[1] The probable error of the mean is calculated in all cases by Peter's formula, and expressed as per cent. of the mean. Thus, if the readings are 35, 40, 40, 40, 45, 40 seconds, the mean time is 40 secs., the sum of the deviations 10, and the probable error of the mean, $\frac{10 \times 0{\cdot}0630 \times 100}{40} = 1{\cdot}5$ per cent. of the mean. In the same way, if the readings are 62, 65, 65, 65, the mean is 64·25, and the probable error 0·44 per cent.: 50, 50, 50, 48, 50, mean 48·6, probable error 0·7 per cent.: 60, 60, 65, 62, 60, mean 61·4, probable error 1·1 per cent.: 95, 100, 95, 100, 100, mean 98, probable error 1·03 per cent. These figures are all from actual experiments, and give an indication of the degree of accuracy with which the 'normal' rate of respiration can be measured.

When the normal rate has been determined as described above, a solution of the reagent in use, having the same concentration of indicator, is substituted for the water and the experiment continued.

Care was taken to use only the purest reagents obtainable. For each experiment, 250 c.c. of the required concentration were made up with tap-water, and adjusted, if necessary, to the standard pH value by the addition of traces of NaOH or HCl : all the solutions were kept in vessels of resistance glass, to avoid variation from acid or alkali dissolved out of the glass. All concentrations are expressed in terms of the molecular weight of the solute, and as all were used at the same pH value, it is assumed that they are strictly comparable.

It may be mentioned here that careful comparison between Haas's direct method and Osterhout's (3) shows that, with material such as this, there is no appreciable difference in accuracy, and the direct method has the advantage that readings can be taken immediately on adding the reagent, without the need for waiting till equilibrium is established as in the closed system of the Osterhout apparatus.

It should be pointed out that in all these experiments, where the carbon dioxide output of seedlings is being measured under water, the oxygen supply is less than the normal: since the controls are also under water, the experiments are quite comparable among themselves. The use of numerous small portions of solution also ensures that the concentration of reagent (and of oxygen) shall be the same throughout the experiment.

RESULTS.

I. *Chloroform.*

The concentrations used were the following: 0·06, 0·04, 0·02, 0·01, 0·001, 0·0001 Molar. (0·06 M. is an approximately saturated solution at room temperature.)

With all concentrations used the rate-time curve had the same general shape. The first effect of the anaesthetic was to cause a depression in the rate of carbon dioxide output: after a length of time varying with the concentration the rate returned to normal and then rose rapidly to a maximum. This period of increase was immediately followed by a continuous depression of the rate. The curve falls rapidly from the maximum to about

60 per cent. of the normal, and then flattens out at a practically constant level of 44 per cent. with all concentrations.

These results can be expressed in the following table:

TABLE I.

WHEAT. (Fig. 1.)

	Rate below normal.			*Rate above normal.*			*Rate below normal.*	
Conc. CHCl₃.	*Minimum.*	*Time to reach minimum.*	*Period of sub-normal rate.*	*Maximum.*	*Time to reach maximum.*	*Period of supra-normal rate.*	*Time to reach 60 %.*	*Rate after 2 hours.*
M.	%	min.	min.	%	min.	min.	min.	%
0·06	60	6	12	300	19	15	—	
0·04	50	6	15	260	21	12	35	44
0·02	50	9	39	150	45	10·5	60	
0·01	50	9	50	120	54	9	78	
0·001	50	9	69	112	72	6	87	
0·0001	50	9	87	100	90		112	
RICE. (Fig. 2.)								
0·06	70	5	8·5	300	16	15	35	32
0·04	58	9	24	200	27	12	45	
0·01	50	12	52	120	55	6		

'*Rate*' is expressed as per cent. of the normal rate as defined above.
'*Time*' is reckoned from the beginning of exposure to the anaesthetic.
The figures given are the mean of several experiments.

These results are similar to those obtained by the writer with ether (8), with several important differences. Thus, with ether the first minimum varies according to the concentration of ether.

With 7·3 per cent. (by vol.) ether (0·72 M.), minimum is 70 per cent.
" 3·65 " " " (0·36 M.), " " 48 per cent.
" 1·0 " " " (0·09 M.), " " 36 per cent.

This agrees with the results of Irwin (2) on animal tissues. The second difference is in the possibility of recovery from the anaesthetic, and will be considered in the next section.

Recovery. In order to test the possibility of recovery from the exposure to chloroform, experiments were made in three different ways.

1. The seedlings, after the normal rate of respiration in tap-water was determined in the usual way, were placed in a large volume of 0·04 M. solution of chloroform for various times. At the end of the period the respiration was taken, first in chloroform and then in tap-water, to see if any recovery took place. It was found that after exposures of one, three, or five hours to 0·04 M. chloroform the rate of respiration was reduced to 50 per cent., 34 per cent., and 32 per cent. of the normal respectively, and that no

recovery took place on removing to tap-water, the rate continuing to decline slowly (Fig. 3).

2. Chloroform 0·04 M., 0·02 M., 0·005 M. was used, and the experiment continued with each concentration until the respiration was reduced to 60 per cent. of the normal. On removing to tap-water the rate fell at once to 50 per cent. and continued at that level. No recovery took place (Fig. 4). This is in marked contrast to the effect with ether, where recovery is possible and appears to be complete, even when the respiration has been reduced to 60 per cent.

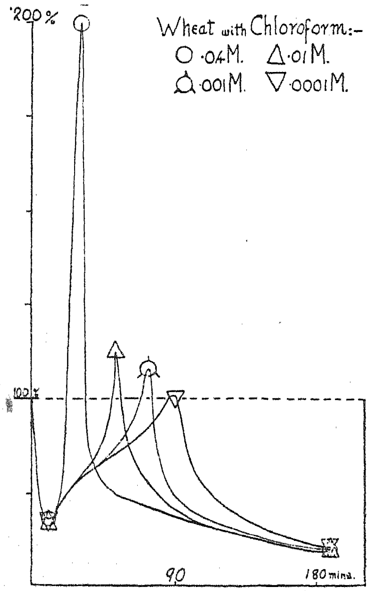

FIG. 1.

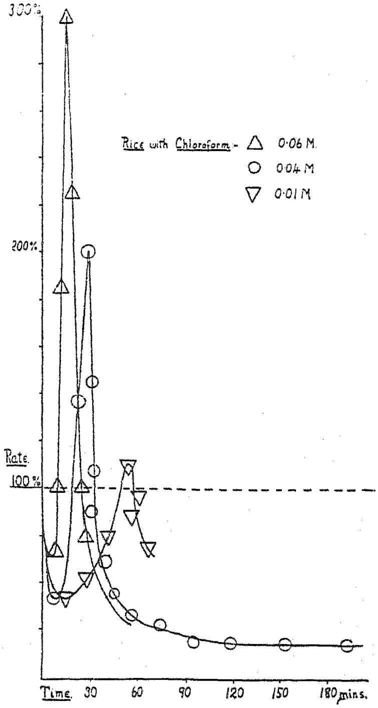

FIG. 2.

FIG. 1. Respiration of wheat with chloroform 0·04 M., 0·01 M., 0·001 M., 0·0001 M. The curve with 0·04 M. is the mean of five experiments (probable error of the mean, less than 4 per cent.): the other curves represent single typical experiments. The normal rate is taken as the reciprocal of the time required to change the pH of 10 c.c. of tap-water from pH 7·36 to pH 7·09, and is expressed as 100 per cent. Deviations from the normal are expressed as per cent. of the normal.

FIG. 2. Respiration of rice with chloroform 0·06 M., 0·04 M., 0·01 M. The curve with 0·06 M. is the mean of three experiments, probable error less than 5 per cent. of mean: with 0·04 M., the mean of six experiments, probable error less than 3·7 per cent.: with 0·01 M., the mean of three experiments, probable error less than 10 per cent. of mean.

3. Chloroform 0·04 M. was used, and the seedlings removed at the first minimum (after six minutes), at the maximum (after twenty minutes), and after twenty-seven minutes, when the rate was again normal. In the first

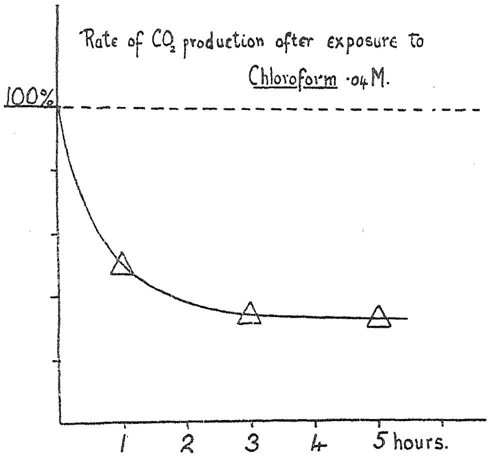

FIG. 3. Rate of carbon dioxide production of wheat after 1, 3, and 5 hours' exposure to chloroform 0·04 M. Three experiments.

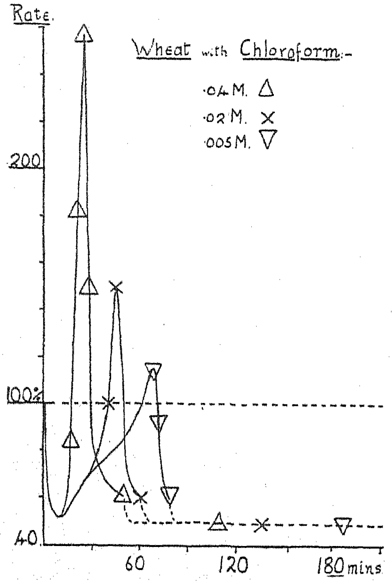

FIG. 4.

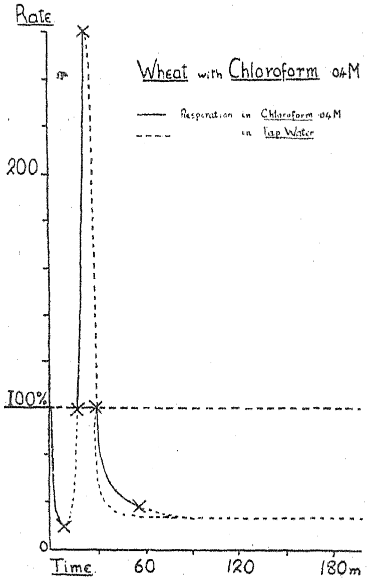

FIG. 5.

FIG. 4. Wheat with chloroform 0·04 M., 0·02 M., 0·005 M., showing that no recovery takes place after the rate has been reduced to 60 per cent., the rate falling to 50 per cent. on removal to tap-water. Solid line, respiration in chloroform; broken line, respiration in tap-water.

FIG. 5. Wheat with chloroform 0·04 M., showing effect of removal to tap-water after 6 minutes, 20 minutes, 27 minutes, and 52 minutes. Recovery only in first case. Composite curve from four experiments.

case the respiration returned to normal and remained so. In the second and third cases the rate fell rapidly to 50 per cent. on removal from the anaesthetic and continued at that level (Fig. 5). A series of experiments

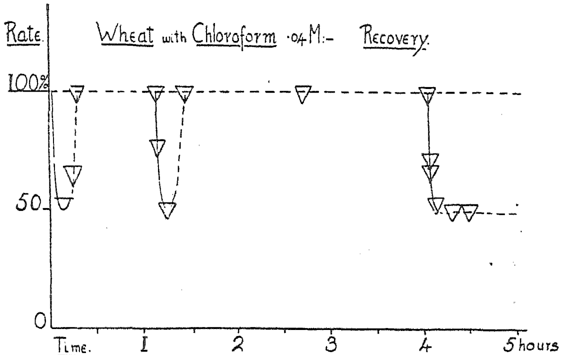

FIG. 6. Wheat with chloroform 0·04 M. Cumulative effect of three exposures of five minutes each, equivalent to fifteen minutes' continuous exposure. Mean of six experiments; probable error of the mean, less than 5 per cent.

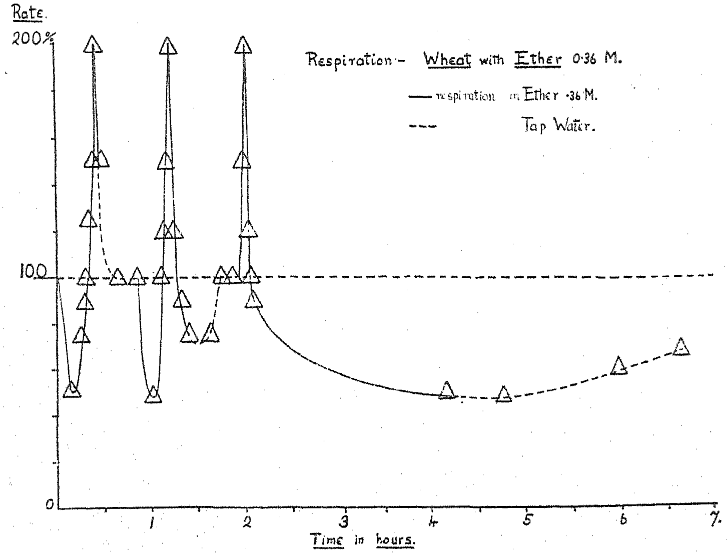

FIG. 7. Wheat with ether 0·36 M., showing three successive exposures to the reagent, the rate being allowed to return to normal (in tap-water) between exposures: the effect is not cumulative. Single experiment.

was then made in which the material was exposed to chloroform (0·04 M.) for three periods of five minutes, with intervals of fifteen minutes in tap-water. Readings were made continuously throughout these experiments in the usual way. After the first two exposures the rate returned to normal: after the third, it fell at once to 50 per cent. on placing in tap-water. Even when the intervals between the exposures were increased to one hour, no recovery was possible after a total exposure of fifteen minutes to the anaesthetic (Fig. 6).

This cumulative effect was not found in ether, where the complete response can be repeatedly evoked, provided the rate of respiration is allowed to return to normal between exposures (Fig. 7).

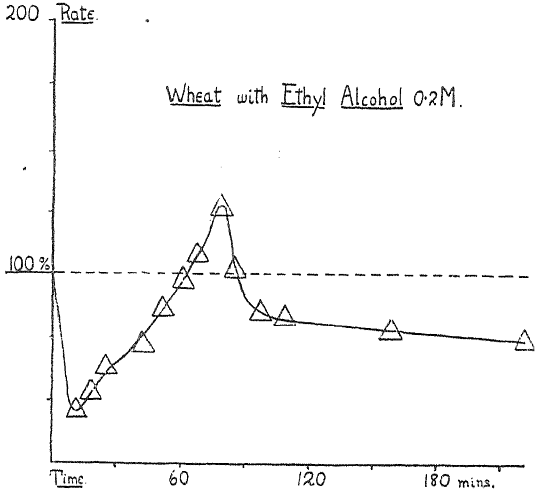

FIG. 8. Respiration of wheat with ethyl alcohol 0·2 M. Mean of four experiments. Probable error, less than 2·5 per cent. of the mean (except for two points, where it is 6 per cent.).

II. *Ethyl Alcohol.*

With ethyl alcohol 0·2 M. the following results were obtained. The rate fell to 60 per cent. in twelve minutes, remaining below normal sixty minutes: rose to a maximum of 125 per cent. in seventy-two minutes, then fell to 75 per cent. in two hours and 64 per cent. in four hours (Fig. 8).

DISCUSSION OF RESULTS.

According to the definition, anaesthesia is reversible: a study of the limits, either of length of exposure or concentration of anaesthetic (the two are of course interdependent), within which recovery can take place, should enable one to define that part of the reaction which can justly be called the 'anaesthetic effect'. Recovery, for present purposes, may be defined as a return of the material to a completely responsive condition. Thus with ether 0·36 M., recovery is possible after six hours' exposure, while with all concentrations of chloroform fifteen minutes seems to be the critical time beyond which recovery will not take place, the rate falling to 50 per cent.

on removal to water in every case (Fig. 4). Whether this means that one-half of the cells of the material are killed, while the rest retain their normal degree of metabolism, or whether all the cells suffer from a permanently lowered metabolism, cannot yet be decided: the fact that after treatment with ether the seedlings can be grown on and will become normally green, while in the case of chloroform no further growth takes place, seems to point to the latter conclusion. It is evident that the chloroform is retained by the cell in some form of combination, either chemical or physical, while ether is almost completely eliminated. This is in accordance with the findings of clinical practice as to the relative toxicity of the two anaesthetics.

It is significant that all concentrations of chloroform from 0·02 M. to 0·0001 M. depress the rate of respiration to 50 per cent. as their first effect.

There are evidently two reactions concerned: one leading to an increased carbon dioxide production, the other to a decreased carbon dioxide production. With low concentrations of chloroform the latter predominates. Only when a certain concentration of chloroform is reached in the cell does the outburst of carbon dioxide occur, and the maximum probably indicates the using up of some antecedent substance, whose formation a low concentration of chloroform hinders. A low concentration acting over a longer time (as with the more dilute solutions) would explain the lowering of the maxima with increasing dilution.

Ray (5) finds that chloroform in low concentrations (0·25 per cent. by vol.) causes an increase in the rate of carbon dioxide production in *Ulva*, followed by a decrease. With 0·5 per cent. only a decrease is observed. In a system consisting of dead tissue (furnishing the oxidizable material), with the addition of H_2O_2 and $Fe_2(SO_4)_3$ as peroxidase, carbon dioxide is given off. The rate of production is affected by chloroform, the exact effect depending on the concentration of iron. If the latter is low, an increase is obtained, followed by a decrease: if high, the rate decreases from the start.

Organic acids oxidized by H_2O_2 and $Fe_2(SO_4)_3$ give off carbon dioxide at a rate which can be measured by the indicator method. In the case of unsaturated acids (such as tannic, oleic, cinnamic) the rate can be affected by chloroform, giving with oleic and cinnamic acids an increase followed by a decrease. With tannic acid, the rate first decreases, then rises above normal, and finally falls (6). From these results he concludes that the effect of chloroform is solely on the oxidative system of the cell, and that the question of the lipoid solubility of the anaesthetic or of the destruction of the plasma membrane can be disregarded (7).

The experiments of the writer do not agree with this conclusion. The differences in the shape of the curve are most probably connected with the presence or absence of oxidizable reserves in the tissues. A plant such as *Ulva*, where there can be no great quantity of reserve material available, might, when treated with 0·5 per cent. (about 0·06 M.) chloroform, use up the substance antecedent to carbon dioxide so quickly that a preliminary outburst of carbon dioxide might be over before the first reading could be made.

Moreover, the experiments on recovery show that the chloroform enters into a more stable association with some cell constituent than would be the case if it merely acted as a catalyst, as is suggested by Ray (7), on the analogy that chloroform accelerates the production of carbon dioxide at low concentrations, hinders it at higher ones, as certain acids affect enzyme action.

There is yet another possibility which must be taken into account. The writer has found, using the corolla of *Ipomoea Learii*, that chloroform and ether markedly decrease the permeability of the cell to carbon dioxide (9). The decreased permeability lasted for fifteen to twenty minutes, and was followed by an equally striking increase which rapidly approached infinity. Of course, the permeability never actually became infinite—or, to put it another way, the time taken for the carbon dioxide to penetrate into the cell was always measurable, because even when the tissue was killed (as seen by colour changes) the cell-walls still presented a residual resistance to the passage of carbon dioxide. On plotting the respiration rate-time curve side by side with the permeability-to-carbon-dioxide-time curve, it appears that the duration of decreased permeability practically coincides with the duration of the subnormal rate of carbon dioxide production (Fig. 9). Whether this time-relation holds for other types of tissues remains to be seen. Should it prove to be so, a new interpretation of these respiration curves may be required. It is offered as a tentative suggestion that they are really composite, being the resultant of two effects: that of the anaesthetic on respiration proper, and on the permeability of the cell to carbon dioxide. It may appear that the true anaesthetic effect is a profound depression of respiration, and that the apparent outburst of carbon dioxide at the maximum may simply be the release of carbon dioxide retained in the cell during the period of decreased permeability, and not an increase in respiration. The effect of the very dilute solutions used seems to point in this direction.

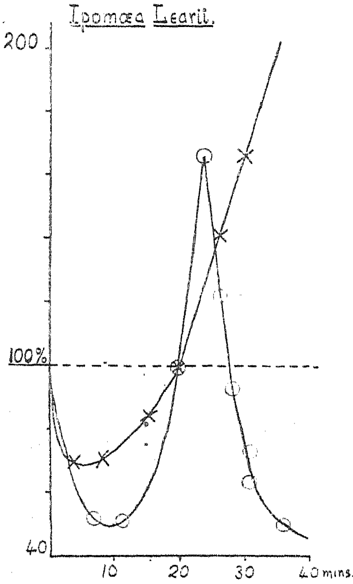

FIG. 9. *Ipomoea Learii.* Respiration (curve drawn through circles), and permeability to carbon dioxide (curve drawn through crosses), as effected by 0·04 M. chloroform. Normal in both cases is expressed as 100 per cent. (For method of determining permeability, see (9)). Each curve is the mean of three typical experiments.

SUMMARY.

1. The effects of various concentrations of chloroform, ether, and ethyl alcohol upon the carbon dioxide output of wheat, rice, and oats, are compared.

2. It is found that these substances, while differing widely in chemical constitution, have similar effects on the respiration of the material used; the first effect is a decrease in the rate of respiration, which is followed by an increase to a maximum and a final depression.

3. These results differ from those obtained by Ray (5) on *Ulva*, probably due to differences in the oxidizable material available in the tissues.

4. Recovery (defined as a return of the material to a completely responsive state) is only possible after very short (less than fifteen minutes) exposure to chloroform, and this whether the exposure is continuous or intermittent. With ether, recovery can take place even after six hours' exposure, provided the respiration is not allowed to fall below 60 per cent.

5. It is concluded that chloroform enters into a more stable combination with some cell constituent than ether, and that the differences in toxicity of these anaesthetics may be due to that cause.

6. Experiments with *Ipomoea* indicate that the effect of the anaesthetic upon the permeability of the plasma membrane to carbon dioxide must be taken into account in the interpretation of these results.

The experimental work upon which this paper is based was begun in the Harvard Laboratory of Plant Physiology, under the direction of Professor W. J. V. Osterhout, to whom the writer wishes to express her thanks. It was continued in the Department of Plant Physiology, the Royal Botanic Garden, Edinburgh. The writer wishes to express to the Regius Keeper, Professor W. Wright Smith, and to Dr. R. J. D. Graham, her appreciation of their great kindness in placing the resources of the Department at her disposal.

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Contributions to an Investigation of the Chemical Nature of the Cellulose Membrane.

BY

F. M. WOOD, B.Sc., F.I.C.

AT an early stage in the study of the chemical nature of the cellulose membrane begun in 1921, it was found necessary to undertake a re-investigation of the methods of preparation and staining in order to differentiate adequately between cellulose and pectic compounds. The results are given below.

The experiments have been conducted both on fresh and fixed material, and the plants used include young root-tips of *Vicia Faba* and *Helianthus annuus*, and certain varieties of *Galtonia*, *Hyacinthus*, &c., as well as the parenchymatous tissues of the stems of these plants. The tissues were chosen as most likely to contain the constituents of the cell-wall in form nearest to that in which they were laid down after cell-division had taken place.

The root-tips were cut off three or four millimetres above the root-cap, so as to include that region where dividing cells are to be found, and both longitudinal and transverse sections were studied. The young stems used were cut also longitudinally and transversely.

When dealing with fixed material the fixative was chosen with a view to producing maximum hydration and minimum oxidation of the cellulose, for this condition should produce maximum differentiation in double-staining for cellulose and pectin, since oxycellulose resembles pectin in its colour reactions more nearly than hydrocellulose.

Hence a known chemical state of the 'labile aggregate' (1) of the cellulose was produced, and attempts to deduce the nature of the cellulose occurring in the cell-wall, before fixation took place, were made.

Could the living cell be killed and fixed under the identical conditions prevailing in life, the solution of the problem would be infinitely easier; but death means chemical change, and this chemical change varies in intensity and kind according to the conditions under which death takes place.

It must not be forgotten that the mere application of the staining reagents used to show the nature of the membrane kills in the majority of

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cases. In dealing with organic compounds, particularly those of an allied nature, partial conversion of one type into another is effected easily, and it is often difficult to distinguish between these types.

In a study of the cell-wall it is desired to distinguish between cellulose and pectic compounds.

Pectocelluloses (pectic compounds) differ from cellulose by an increased proportion of oxygen; they are acid in character and are gelatinizable. They are converted into pectins by dilute mineral acids, and precipitated, whereas cellulose is converted either into hydrocellulose (by dilute sulphuric, hydrochloric, or phosphoric acid) or oxycellulose (by dilute nitric, chromic, or osmic acid).

Caustic alkalis, on the other hand, have little action on cellulose, whilst pectocelluloses are dissolved by them, so that the *chemical* differences between pectic compounds and cellulose are not many, and since these substances may consist of pectocellulose (i. e. cellulose + pectin), a reagent may dissolve out the pectin and leave the cellulose, giving a false idea of the absence of this substance.

It is evident, when using fixed material, that the choice of a fixative is an important matter. As the only means of chemical analysis of tissues is by double-staining, care must be taken that the results obtained really represent the condition of affairs existing in the untreated cell-walls.

For instance, since pectins are precipitated by acids and dissolved by caustic alkalis, any treatment given to the tissues should be acidic rather than alkaline in character, otherwise results obtained by double-staining will depend upon whether the alkaline treatment has been sufficient to remove all the pectin or not.

Most of the usual fixatives are powerful oxidizing agents, and frequently contain metallic radicals. Chromic acid, for example, is a constituent of Flemming's fluid, and contains the metallic radical chromium, and although the solutions used are chemically weak, a certain proportion of the cellulose suffers oxidation, with the result that oxycellulose is produced and predominates, so it is useless to attempt double-staining for cellulose and pectin, since oxycellulose will react with many of the pectin stains.

Even in the living cell we are not dealing with a 'cellulose' cell-wall, but with one which consists as well of derivatives of cellulose—oxycellulose or hydrocellulose. Because of the resemblance of oxycellulose to pectin in its staining reactions, it was found best to perform qualitative tests upon fresh material, in order to determine whether there was much oxycellulose present. The results of these tests as applied to *Vicia Faba* (stem and root), *Helianthus* (stem), and *Hyacinthus* (root) are shown in Table I.

The method used was adapted from that of Knaggs (2) for cotton fibres. The material is washed with dilute hydrochloric acid, then with water, dyed deeply with benzopurpurin, and washed again with acid, when a blue colour is produced. It is then washed in water, when that part of the cell-wall which is not oxidized becomes red, but the oxidized part remains blue-black in colour, the whole having a bluish red appearance.

The persistence of this colour on washing instead of the reappearance of clear red is evidence of the presence of an amount of oxycellulose sufficient to affect double-staining appreciably.

For the detection of oxycellulose in the presence of hydrocellulose and cellulose, Schwalbe's (3) method was adopted. Sections are immersed in methyl-orange and afterwards in concentrated brine, when oxycellulose becomes a deep red, hydrocellulose and ordinary cellulose remaining yellow.

In addition to tests on fresh material Table I shows the reactions produced after treatment with a number of fixatives, and this serves to show the effect of the fixing fluids on the cell-walls of the plants indicated.

The presence of a metallic radical in the fixative may result in the stains not entering into combination with the cellulose or pectin at all, but merely reacting with the metallic radical retained by the plant tissue; for such a substance enters into chemical reaction with the protoplasm and cell-wall, forming compounds which remain after washing. Thus, after the use of the common fixative chrom-acetic acid, which contains the metal chromium, a reaction apparently characteristic of cellulose may be due, not to the cellulose, but to a compound of the dye with chromium, the material of the cell-wall acting as a vehicle to retain it. This fixative has other disadvantages; the acetic acid converts part of the cellulose into cellulose acetate, whether it occurs normally, oxidized, or as hydrocellulose, so no information as to the original state of the membrane is obtained. Besides this, the chromic acid converts part of the cellulose into oxycellulose.

In order to distinguish cellulose from pectin, it is desirable to have the cellulose as much as possible in the form of hydrocellulose, the hydration being controlled, so that the disintegration of the cell-wall that would result if it were entirely converted into hydrocellulose does not occur. Accordingly, a large number of fixatives were used, with varying results. Among these were hydroxylamine hydrochloride, sulphocyanides, oxalic, hydrochloric (one per cent.), and sulphuric (one per cent.) acids, and sodium hyposulphite (4).

Of these hydroxylamine hydrochloride proved the most satisfactory, while hydrochloric and sulphuric acids did not give good results. Hydro-

TABLE I.

Summary of Results obtained using Knaggs's and Schwalbe's tests.

The presence of oxycellulose is shown by the sign (+) and its absence by (–).

Fixative.	*Time Fixing.*	*Oxycellulose as shown by Knaggs's test.*				*Oxycellulose as shown by Schwalbe's test.*			
		Vicia Faba.		*Helianthus.*	*Hyacinthus.*	*Vicia Faba.*		*Helianthus.*	*Hyacinthus.*
		Stem.	*Root.*	*Stem.*	*Root.*	*Stem.*	*Root.*	*Stem.*	*Root.*
Unfixed (control experiments).	–	–	–	–	–	– + in phloem, sclerenchyma, and xylem.	– + in phloem, sclerenchyma, and xylem.	– + in outer layers of cortex.	– + in outer layers of cortex.
Hydroxylamine hydrochloride.	15 mins.	+	+	–	–	– + in phloem, sclerenchyma, and xylem.	– + in phloem, sclerenchyma, and xylem.	– + in phloem, xylem, and sclerenchyma.	– + in outer layers of cortex.
Ammonium sulphocyanide.	15 mins.	–	Slightly +	Slightly + in stele.	–	– + in phloem, sclerenchyma, and xylem.	– + in phloem, outer layers of cortex, and xylem.	+	–
Hydrochloric acid.	10 mins.	Slightly +	Slightly +	Very slightly +	– slightly + in outer layers of cortex.	+ Strong oxycellulose reaction in sclerenchyma and phloem.	+ Strong oxycellulose reaction in phloem.	+	–
Sulphuric acid.	10 mins.	Slightly +	Slightly +	Very slightly +	+	+ Strong oxycellulose reaction in sclerenchyma.	+	+	+

Sodium hypo sulphite.	10 mins.	+ much.	+ much.	+	+	— + in phloem fibres.	— + in phloem and xylem.	—	— but some slight oxycellulose effect.
Hydrogen peroxide.	15 mins.	+ slight.	+ slight.	+ very slight.	+	— + in phloem, xylem, and tissues round stele.	— + in outer layers of cortex.	—	— + in outer layers of cortex.
Calcium hypochlorite.	15 mins.	— but trace in stele.	— but trace in stele.	—	+ except in stele.	+ Strong oxycellulose reaction in stele.	+ Strong oxycellulose reaction in stele.	Uncertain slight oxycellulose reaction.	— mainly, but a trace of oxycellulose.
Oxalic acid.	10 mins.	+	+	+	+ brownish blue.	All cell-walls yellowish brown. Oxycellulose in phloem.	All cell-walls yellowish brown.	All cell-walls yellowish brown.	Brown effect, possibly both bodies present.
Potassium ferrocyanide.	15 mins.	+ slight.	+ slight.	— but trace in stele.	+ especially cortex.	— + in phloem fibres.	— except in phloem fibres.	—	— Outer layers of cortex and pericycle yellow.
Hydrocyanic acid.	10 mins.	+	+	+	+	— except in sclerenchyma.	+	—	—
Potassium cyanide.	10 mins.	+ slight.	+	+	—	— except in phloem fibres.	—	—	—
Sodium arsenate.	10 mins.	+	+	—	+	+ Especially strong oxycellulose reaction in stele.	+ Strong oxycellulose reaction in stele.	—	— all tissues.
Phenol.	10 mins.	+ slight.	+	—	+	+ Stele shows oxycellulose reaction strongly.	+ Stele shows oxycellulose reaction strongly.	—	— very yellow

TABLE I (*continued*).

Fixative.	*Time Fixing.*	*Oxycellulose as shown by Knaggs's test.*				*Oxycellulose as shown by Schwalbe's test.*			
		Vicia Faba.		*Helianthus.*	*Hyacinthus.*	*Vicia Faba.*		*Helianthus.*	*Hyacinthus.*
		Stem.	*Root.*	*Stem.*	*Root.*	*Stem.*	+ *Root.*	*Stem.*	*Root.*
Chrome-acetic.	8 mins.	+ much.	+ much.	+	+	– only a slight + reaction in phloem.		–	+ slightly oxidized.
Phenol and acetic acid.	10 mins.	+	+ in stele only.	–	– except in outer layer of cortex.	+ but – in pith-cells.	+ but – in pith-cells.	–	– except outer layers of cortex.
Nitric acid (concentrated).	2 mins.	+	+	+	+ disintegrated.	+	+	+	+ disintegrated.
Cresol.	10 mins.	+ slight.	+ slight.	–	–	– except in phloem fibres.	–	–	– Outer layers of cortex bright yellow.

xylamine hydrochloride, however, is not suitable for material which has to be double-stained, because a little alcohol must be used in order to effect solution, and this, as described later, may interfere with the pectin stains. The other fixatives mentioned fall between these two classes.

The method adopted was to cut a section by hand from fresh material, and watch under the microscope the action of the fixative upon the cells, the section being immersed in the fixative on a hollow slide.

By way of contrast, hydrogen peroxide and calcium hypochlorite were used, the object being to produce oxycellulose (5).

In no case was the result satisfactory for staining, and in many instances partial disintegration of the cells took place. With hydrogen peroxide the shape of the cells was well preserved, but the contents were very much disintegrated, and the wall was inclined to react with pectin stains, showing that it had been partially converted into oxycellulose.

Calcium hypochlorite was used to accentuate the pectin, since calcium salts will coagulate pectates, but it was unsatisfactory for the cellulose.

In order to mordant the stain upon the cell-wall, and to combine with this, a powerful poison, potassium ferrocyanide, was used. This was fairly satisfactory, but it is doubtful whether there is anything to be gained by the introduction of the metallic radical.

Hydrocyanic acid proved satisfactory, but potassium cyanide was not suitable.

Sodium arsenate was found unsuitable for fixation, and apparently prevented the cell-wall from absorbing stains. The lack of success with sodium and potassium salts is attributed to the presence of these metallic radicals, rather than to the remaining portion of the molecules.

Carbolic acid and cresol were also used, and worked fairly well, though each was inclined to disintegrate the cell contents unduly. These substances would have little effect upon the cellulose in the cell-wall, as they do not react with cellulose in weak solution in the cold.

In no case did it prove advisable to use any fixative in a concentration greater than 5 per cent.; this should be preferably from 2 to 4 per cent., otherwise most of the fixatives mentioned cause distortion and some disintegration of the cell. The general effects of these fixatives on the cellulose cell-wall in the plants studied are summarized in Table II.

It is necessary to avoid alcohol and glycerine when fixed material is prepared for staining. Either of these substances will wash out pectin stains (7, 8), and should a trace of either remain in the tissue, the result of differential staining is without significance. Alcohol is objectionable because it is obstinately retained by cellulose, and is not removable by heat (9).

Fresh material treated with alcohol, however well washed, is liable

TABLE II.

Fixatives.

Fixative.	*Strength.*	*Effect on Cellulose.*	*Effect on Pectic Compounds.*	*Results and Remarks.*
Hydroxylamine hydrochloride.	1·8 %.	Hydrocellulose produced in some tissues. (See Table I.)	Pectin precipitated.	A fairly good fixative, but as a little alcohol has to be used in order to effect solution, *double*-staining would be uncertain after its use.
Ammonium sulphocyanide.	2 %.	Hydrocellulose. (See Table I.)	Apparently there is little action.	A moderately good fixative; nuclei show up well.
Hydrochloric acid.	1 %.	Hydrocellulose (4) and oxycellulose. (Table I.)	Pectin precipitated.	Not a good fixative for the cell as a whole.
Sulphuric acid.	1 %.	Hydrocellulose (4) and oxycellulose. (Table I.)	Pectin precipitated.	„
Sodium hyposulphite.	2 %.	Hydrocellulose and some oxycellulose. (Table I.)	Little effect.	Staining after this was not 'clean'.
Hydrogen peroxide.	5 c.c. of 10 volumes concentration were diluted to 25 c.c. with water.	Oxycellulose (5). (See Table I.)	No effect.	Double-staining would not show up, owing to oxycellulose formed.
Calcium hypochlorite.	2½ %.	Oxycellulose (5), but little shown to be present by Table I.	Effect on pectic compounds was masked by oxycellulose.	Distortion occurs at first, but afterwards there is some recovery from this.
Oxalic acid.	3-5 %.	Hydrocellulose (3) and oxycellulose. (Table I.)	Pectin was precipitated.	This was not found suitable owing to the distortion caused.
Potassium ferrocyanide.	2 %.	—	—	No proper double-staining resulted, so the effect could not be traced.
Hydrocyanic acid.	About 0·5 %.	Possibly some hydrocellulose produced.	Pectin was precipitated.	Apparently good—there was little distortion of the cells.
Potassium cyanide.	2 %.	—	—	This was not at all satisfactory.
Sodium arsenate.	2 %.	Both oxycellulose and hydrocellulose produced. (Table I.)		This seemed to spoil the cell-wall for all staining reactions. It was found unsuitable.
Phenol (Carbolic acid).	1 %, 2½ %, 3 %.	„	—	A good fixative for the cell-wall, but it seems to destroy protoplasmic structures.
Cresol ('Lysol').	0·5 c.c. of the concentrated Lysol diluted to 100 c.c.	„	—	A fairly satisfactory fixative, but some slight distortion.

to contain a little, and the temperature (50°–60° C.) employed during embedding fixed material in paraffin is not sufficient to remove it. Indeed, if a sufficiently high temperature were employed (say 100° C. or even higher) a profound modification of the cellulose would occur.

Glycerine was found still present in cellulose material after washing for three days in running water. The practice in vogue in some laboratories (8, 10) of treating tissues with acid alcohol before staining may produce misleading results owing to the retained alcohol interfering with the pectin stains.

Where fixed material was embedded in paraffin and cut by means of the microtome, alcohol was entirely excluded, the slide to which the sections were attached being dried, after immersion in xylol, at about 55° C.

In staining, water was removed by drying instead of by the use of alcohol.

To demonstrate the presence of pectin in the cell-wall appropriate double stains were used.

To classify these dyes according to their chemical constitution is a somewhat difficult task. Mangin (11) arranged them according to certain complex organic groups; this classification, though enlightening to a chemist, may seem somewhat complicated to others.

It has been found that a simpler form of chemical classification can be adopted, and it is hoped, by this means, to show which chemical groupings are really concerned with the absorption of dyes by pectin and cellulose, and so to throw further light upon the mechanism of the reactions that take place, and hence upon the true nature of the cell-wall.

So far as the present investigation goes, the organic stains used for this purpose fall into two groups:

(1) Hydrochlorides of amines (or substituted amines) [$—NH_2.Cl$ or $—NH_2.HCl$]. In this group are found all the pectin stains.

(2) Alkali salts of the disulphonic acids of compounds containing one or more azo-groups [$—SO_3Na$ and $—N = N—$ groupings].

Table III contains a list of most of the staining reagents for cellulose, and Table IV those for pectin, all chemical nomenclature and formulae being in accordance with the 'Colour Index' of the Society of Dyers and Colourists (12).

In many instances the colour reactions cited also serve to distinguish the pectin or cellulose from callose, suberin, or lignin.

For the sake of convenience, in the formulae given, the significant parts of the molecules have been separated from the remainder and placed at the end regardless of the molecular constitution.

Pectin, therefore, appears to have an attraction for the amino ($—NH_2$) group, while cellulose attracts the sulphonic acid ($—SO_3H$) group when it is in association with one or more azo-groups ($—N = N—$).

TABLE III.

Dyes staining Cellulose.

Alkali salts of Disulphonic Acids of Compounds containing two Azo-groups.

Dye.	*Scientific Name.*	*Formula.*
(*A*) *Two Azo-groups and two Sulphonic Acid Groups.*		$[(—N = N—)_2$ and $(SO_3Na)_2.]$
Crocein.	Sodium salt of benzene-azo-benzene-α-naphthol-3 : 6-disulphonic acid.	$C_{22}H_{14}O—N_4(SO_3Na)_2$.
Congo red.	Sodium salt of diphenyl-disazo-bis-α-naphthylamine-4-sulphonic acid.	$C_{32}N_2—N_4(SO_3Na)_2$.
Congo G. R.	Sodium salt of diphenyl-diazoamino-*m*-sulpho-benzene-azo-naphthylamine-4-sulphonic acid.	$C_{28}H_{20}N_2—N_4(SO_3Na)_2$.
Congo Corinth.	Sodium salt of diphenyl-disazo-4-sulpho-α-naphthylamine-α-naphthol-4-sulphonic acid.	$C_{32}H_{21}N.O—N_4(SO_3Na)_2$.
Congo Corinth B.	Sodium salt of ditolyl-disazo-4-sulpho-α-naphthylamine-α-naphthol-4-sulphonic acid.	$C_{34}H_{25}S_2NO—N_4(SO_3Na)_2$.
Azo blue.	Sodium salt of ditolyl-disazo-bis-α-naphthol-4-sulphonic acid.	$C_{34}H_{24}O_2—N_4(SO_3Na)_2$.
Azo violet.	Sodium salt of dimethoxydiphenyl-disazo-4-sulpho-α-naphthylamine-α-naphthol-4-sulphonic acid.	$C_{34}H_{25}NO_3—N_4(SO_3Na)_2$.
Heliotrope.	Sodium salt of dimethoxydiphenyl-disazo-bis-ethyl-β-naphthylamine-7-sulphonic acid.	$C_{38}H_{34}N_2O_2—N_4(SO_3Na)_2$.
Naphthol black.	Sodium salt of *p*-nitrobenzene-azo-3 : 6-disulpho-1-amino-8-naphthol-azo-benzene.	$C_{22}H_{14}N_2O_3—N_4(SO_3Na)_2$.
Bordeaux extra (Congo violet).	Sodium salt of diphenyl-disazo-bis-β-naphthol-8-sulphonic acid.	$C_{32}H_{20}O_2—N_4(SO_3Na)_2$.
Deltapurpurine.	Sodium salt of ditolyl-disazo-bis-β-naphthylamine-7-sulphonic acid.	$C_{34}H_{26}N_2—N_4(So_3Na)_2$.
Benzopurpurine.	Sodium salt of ditolyl-disazo-bis-α-naphthylamine-4-sulphonic acid.	$C_{34}H_{26}N_2—N_4(SO_3Na)_2$.
Orseilline BB.	Sodium salt of *p*-sulpho-*o*-toluene-azo-*o*-toluene-azo-α-naphthol-4-sulphonic acid.	$C_{24}H_{18}O—N_4(SO_3Na)_2$.
Orseille red (Orchil red A).	Sodium salt of *m*-xylene-azo-*m*-xylene-azo-β-naphthol-3 : 6-disulphonic acid.	$C_{26}H_{22}O—N_4(SO_3Na)_2$.
(*B*) *Two Azo-groups and three Sulphonic Acid Groups.*		$[(—N = N—)_2$ and $(SO_3Na)_3.]$
Brilliant Congo G.	Sodium salt of diphenyl-disazo-6-sulpho-β-naphthylamine-β-naphthylamine-3 : 6-disulphonic acid.	$C_{32}H_{21}N_2—N_4(SO_3Na)_3$.
(*C*) *One Azo-group and two Sulphonic Acid Groups.*		$[(—N = N—)$ and $(SO_3Na)_2.]$
Ponceaux.	Sodium salt of toluene-azo-β-naphthol-3 : 6-disulphonic acid.	$C_{17}H_{12}O—N_2(SO_3Na)_2$.
Azo-rubine.	Sodium salt of 4-sulpho-α-naphthalene-azo-α-naphthol-4-sulphonic acid.	$C_{20}H_{12}O—N_2(SO_3Na)_2$.

TABLE IV.

Dyes staining Pectic Compounds.

Hydrochlorides of Amines.

Dye.	*Scientific Name.*	*Formula.*
(*A*) *Containing one Amino Group in the Molecule.*		[—(NH_2) and Cl or HCl.]
Chrysoidine.	Hydrochloride of benzene-azo-*m*-phenylenediamine or *m*-di-aminoazo-benzene.	$C_{12}H_{10}N_2—(NH_2)HCl$.
Auramine.	Hydrochloride of tetramethyl-diamino-diphenyl ketonimine.	$C_{17}H_{20}N_2—(NH)HCl$.
Magdala red.	Mixture of amino-naphthyl-dinaphthazonium chloride and diamino-naphthyl-dinaphthazonium chloride.	$C_{30}H_{18}N_2—NH_2—Cl$.
Neutral blue.	Dimethylamino-phenyl-naphtho-phenazonium chloride or dimethyl-*iso*-rosinduline chloride.	$C_{22}H_{14}N_2—N(CH_3)_2—Cl$.
Naphthalene blue.	Dimethylamino-naphtho-phenazoxonium chloride, or its zinc double chloride.	$C_{16}H_9NO—N(CH_3)_2—Cl$.
(*B*) *Containing two Amino Groups in the Molecule.*		[—$(NH_2)_2$ and Cl or HCl.]
Phenosafranin.	Diamido-phenyl-diphenazonium chloride.	$C_{18}H_{11}.N_2—(NH_2)_2—Cl$.
Safranin.	Mixture of diamino-phenyl-ditolazonium chloride and diamino-*o*-tolyl-ditolazonium chloride.	$C_{20}H_{15}.N_2—(NH_2)_2—Cl$. $C_{21}H_{17}.N_2—(NH_2)_2—Cl$.
Nile blue 2B.	Diethylamino-benzylamino-naphtho-phenoxonium chloride.	$C_{16}H_8NO—NH(CH_2.C_6H_5).N(C_2H_5)_3Cl$.
Rosolane (Mauveine).	Mainly amino-phenyl-amino-*p*-tolyl-ditolazonium sulphate, together with lower homologues.	$C_{27}H_{22}.N_2—NH.NH_2—(SO_4)\frac{1}{2}$.
Methylene blue.	Tetramethyl-diamino-diphenazthionium chloride.	$C_{12}H_6N.S—[N(CH_3)_2]_2Cl$.
(*C*) *Containing three Amino Groups in the Molecule.*		[—$(NH_2)_3$ and Cl or HCl.]
Methyl violet.	Mixture of the hydrochlorides of the more highly oxidized methylated pararosanilines, containing principally tetra, penta, and hexamethyl derivatives.	$C_{19}H_{12}—NH(CH_3)[N(CH_3)_2]_2—Cl$.
Hoffmann's violet.	A mixture of methylated or ethylated rosanilines and pararosanilines of varying composition.	$C_{20}H_{14}—[NH.(C_2H_5)]_3Cl$.
Fuchsin (and many of its derivations, i. e. Aurine).	Mixture of pararosaniline and rosaniline hydrochlorides.	$C_{20}H_{14}—(NH_2)_3$ Cl.
Victoria blue.	Hydrochloride of tetramethyl-phenyl-triamino-diphenyl-α-naphthyl-carbinol anhydride.	$C_{37}H_{19}—NH.[N(CH_3)_2]_2Cl$.
Night blue.	Hydrochloride of tetramethyl-*p*-tolyltri-amino-diphenyl-α-naphthyl-carbinol anhydride.	$C_{31}H_{21}—N.H.[N(C_2H_5)_2]_2Cl$.
(*D*) *Containing four Amino Groups in the Molecule.*		[—$(NH_2)_4$ and Cl or HCL.]
Bismarck brown.	Hydrochloride of benzene-*m*-disazo-bis-phenelene-diamine.	$C_{18}H_{10}N_4—(NH_2)_2(NH_2.HCl)_2$.

Mangin (11) records that cellulose is stained more deeply the greater the sulphonation. Green and Saunders (14) state: 'Since the discovery of the alkali and soluble blues by Nicholson in 1862, the almost exclusive method of conferring solubility and acid dyeing properties upon synthetic colouring matters has been by sulphonation, that is, by the introduction of sulphonic acid groups (—HSO_3) into the hydrocarbon nucleus.'

There are one or two comments to be made upon Tables III and IV.

Ponceaux and azo-rubine are stated by Mangin (11) to stain cellulose, but only contain one azo-group. However, the disulphonic acid groups appear to compensate for this. Rosolane, a pectin stain (11), is not a hydrochloride, but contains a $\frac{1}{2}(SO_4)$ group. If there is any 'damping' effect due to this it seems to be overcome by the presence of the two amino groups.

In these experiments methylene blue was found a somewhat uncertain stain with which to work. This may be due to the presence in the molecule of a sulphur atom which does not occur in any of the other pectin stains.

Sulphur has been shown by Knecht and Thompson (15) to become fixed on the cotton fibre, from which it is not removable by washing with boiling water or alkali.

Sulphuric-acid-treated cotton contains fixed sulphur, and little if any oxycellulose.

This points to sulphuric acid being an unsuitable fixative for the purpose of this investigation.

It would be interesting to discover whether the presence of sulphur in the methylene blue molecule is, in the process of staining, capable of being fixed in any way upon the cellulose, and so cause varying results.

Working upon these lines it has been found best to attempt to produce a certain amount of 'hydrocellulose' when fixing, as this form of cellulose works better in double-staining.

Evidently the good staining results of other investigators (8, 10) after the use of acid alcohol (hydrochloric acid 1 part, and 4 to 5 parts of alcohol) were due to the formation of hydrocellulose, but, as pointed out already, no certainty of results with pectin stains could be relied upon after the use of this reagent.

The reliability of ruthenium red (6, 7, 8) as a stain for pectin depends upon its inability to be washed out by either glycerine or alcohol. Being an inorganic dye, this cannot be placed in the same category as the others.

Tables V and VI give a *résumé* of some of the results obtained with freehand sections and with material embedded in paraffin. The sections were, in the majority of cases, longitudinal ones, and the results recorded refer to tissues with cellulose cell-walls. It is from the results given in

TABLE V.

Fixation and Staining of Helianthus annuus Stem (Freehand Sections).

					Stains used.		
Fixative.	*Time Fixing.*	*Cell Distortion.*	*Cell-wall.*	*Protoplasm.*	*For Cellulose.*	*For Pectin.*	*Effect of Staining.*
Hydroxylamine hydrochloride.	15 mins.	None—slight general shrinkage.	Became thinner, 'lined', and emphasized.	Not contracted or precipitated.	Crocein.	—	Cell-walls absorbed the stain more readily than unfixed ones.
Ammonium sulphocyanide.	3 mins.	Slight.	Slightly 'lined'.	Nuclei especially clear.	Crocein.	Methyl violet.	Cell-walls only slightly stained yellow with crocein.
Hydrochloric acid.	5 mins.	Slight.	Tendency towards disintegration.	Hardly affected.	Crocein.	Methyl violet.	Only slight staining of the cell-wall with crocein.
Sulphuric acid.	5 mins.	Slight.	Tendency towards disintegration.	Hardly affected.	Crocein.	Methyl violet.	Only faint staining of the cell-wall with crocein.
Sodium hyposulphite.	10 mins.	Slight. Lumen not decreased.	Became slightly 'lined' and outline irregular.	Nuclei swelled up.	Benzopurpurin.	Methylene blue.	The whole section stained reddish purple.
Hydrogen peroxide.	2–3 mins.	None.	Emphasized at once.	Greatly affected.	Crocein.	Methyl violet.	No staining with crocein.
Calcium hypochlorite.	2–3 mins.	Fair amount of distortion. Lumen contracted.	'Lined' and slightly disintegrated.	Precipitated. Granules driven to the sides of the cell.	Crocein.	Methylene blue.	Crocein is not absorbed well.
Oxalic acid.	10 mins.	Distorted.	Not swollen or broken.	Precipitated and collected at sides of cell.	Orseille red.	Methyl violet.	Orseille red did not stain well. Nuclei were purple.
Potassium ferrocyanide.	2–3 mins.	Slight. Lumen not decreased.	No 'lining'. Pith cells disintegrated.	Slightly contracted.	Benzopurpurin.	Methylene blue.	Benzopurpurin was only slightly absorbed.
Hydrocyanic acid.	2–3 mins.	None.	Unaffected.	Slightly contracted.	Orseille red.	Methyl violet.	Methyl violet was not absorbed at all.
Potassium cyanide.	2-3 mins.	Slight.	Softened and outline irregular.	Precipitated.	Crocein.	Methyl violet.	Neither stain was absorbed properly.
Sodium arsenate.	2–3 mins.	Slight. Lumen decreased.	Became much thinner.	Slightly affected.	Crocein.	Methyl violet.	Neither stain was absorbed properly.
Phenol.	3 mins.	None.	Unaffected.	Unduly affected and precipitated.	Crocein.	Methyl violet.	Very slight yellow colour in cell-walls.

TABLE V—(*continued*).

Fixing and Staining of Helianthus annuus Stem (Freehand Sections).

					Stains used.		
Fixative.	*Time Fixing.*	*Cell Distortion.*	*Cell-wall.*	*Protoplasm.*	*For Cellulose.*	*For Pectin.*	*Effect of Staining.*
Phenol and acetic acid.	3 mins.	None.	Unaffected.	Nuclei became very distinct.	Crocein.	Methyl violet.	Cell-walls take crocein faintly, but better than unfixed material.
Cresol.	3 mins.	Slight.	Unaffected.	Slightly precipitated.	Crocein.	Methyl violet.	Staining very poor. Nuclei purple.
Chrom-acetic (medium strength).	3 mins.	None. Lumen of cells decreased.	Thinner and 'lined'.	Not contracted.	Benzopurpurin.	Methylene blue.	All cells were purplish in colour.

Unless otherwise stated, cellulose and pectin were stained by the appropriate dye.

TABLE VI *a*.

Fixation and Staining of Vicia Faba (Root Apex) Embedded Material.

						Staining.		
Fixative.	*Time Fixing.*	*Treatment before Staining.*	*Cell Distortion.*	*Cell-wall.*	*Protoplasm.*	*For Cellulose.*	*For Pectin.*	*Staining Effects.*
Phenol 10 %.	6 hrs. warm.	—	Marked.	Broken up.	Contracted.	Naphthol black.	Phenosafranin.	Poor. Fixative is too concentrated.
		Acid alcohol 24 hrs.	”	”	”	”	”	”
		”	”	”	”	Crocein.	Victoria blue.	Little crocein showing.
		72½ % sulphuric acid (5 mins.).	”	”	”	”	”	” Nuclei blue.
		Saturated ammonium oxalate 24 hrs.	”	Cells separated from one another.	Slightly contracted.	Azo blue.	Fuchsin.	No double-staining.
		Alcoholic potash 24 hrs.	”	Broken up.	Contracted.	Congo red.	Victoria blue.	No blue showing.
Sodium arsenate 10 %.	23½ hrs.	—	”	Broken in places.	Peculiarly contracted.	Naphthol black.	Phenosafranin.	Phenosafranin alone showing; nuclei swollen and very dark.

TABLE VI *a*—(*continued*).

Fixation and Staining of Vicia Faba (Root Apex) Embedded Material.

Fixative.	*Time Fixing.*	*Treatment before Staining.*	*Cell Distortion.*	*Cell-wall.*	*Protoplasm.*	*Staining.*		*Staining Effects.*
						For Cellulose.	*For Pectin.*	
Potassium cyanide 10 %.	24 hrs.	—	Slight.	Sometimes broken.	Contracted.	Orseille red.	Victoria blue.	Very little colour.
		—	,,	,,	,,	Azo blue (clove oil).	Fuchsin.	Cell-walls blue, contents purple.
		—	,,	,,	Peculiarly contracted, but nucleus very distinct.	Naphthol black.	,,	Purple-brown all over.
		Concentrated acetic acid 2 mins.	,,	,,	,,	,,	,,	,,
		Acetic alcohol 24 hrs.	Marked.	Disintegrated.	Disintegrated.	Azo blue.	Bismarck brown.	No double-staining.
		Alcoholic potash 16 hrs.	,,	Broken in many cases.	Contracted.	Congored.	Methyl violet.	Walls violet, contents reddish.
		—	,,	Broken in some cases.	Disintegrated.	Azo blue.	Phenosafranin.	Indecisive.
		Alcoholic potash (a few mins.).	,,	,,	,,	,,	,,	Clearer than the above.
		—	,,	,,	Contracted.	Naphthol black.	Bismarck brown.	No proper double-staining, but some black shows in the walls in a few cases.
		Alcoholic potash.	,,	,,	,,	,,	,,	,,
		Acid alcohol 24 hrs.	,,	Broken up.	,,	Crocein.	Victoria blue.	The whole section was a pale yellow colour.
Potassium ferrocyanide 10 %.	23½ hrs.	—	Much.	Very little.	Precipitated, but not contracted.	Azo blue.	Phenosafranin.	Very deep staining, and only blue showing.

TABLE VI *b*.

Fixation and Staining of Vicia Faba (Root Apex) Embedded Material.

						Staining.		
Fixative.	*Time Fixing.*	*Treatment before Staining.*	*Cell Distortion.*	*Cell-wall.*	*Protoplasm.*	*For Cellulose.*	*For Pectin.*	*Staining Effects.*
Phenol 6 %.	3 hrs.	—	Marked.	Cells separated from one another.	Slightly contracted.	Crocein.	Fuchsin.	Whole section reddish yellow.
		—	None.	Little affected.	Contracted.	Naphthol black.	,,	Cell-walls black, contents red.
		—	Slight.	Unbroken.	Disintegrated.	,,	Phenosafranin.	No proper double-staining.
		Acid alcohol 24 hrs.	Marked.	Broken up.	,,	,,	,,	,,
		72·5 % sulphuric acid 20 secs.	,,	,,	,,	Azo blue.	Fuchsin.	No clear double-staining.
		Alcoholic potash 24 hrs.	,,	,,	,,	Congo red.	Victoria blue.	Whole section red only.
		Acetic alcohol 24 hrs.	,,	,,	,,	Naphthol black.	—	Whole section took the stain fairly well.
Hydrocyanic acid 5 %.	4 hrs.	—	—	Outline slightly wavy.	Often contracted.	Crocein.	Methylene blue.	Cell-walls blue; contents very deeply stained.
		—	—	,,	,,	Naphthol black.	Phenosafranin.	Red generally; nuclei dark purple.
Potassium cyanide 2 %.	2 hrs.	Acid alcohol 24 hrs.	Little or none.	Thickened.	Contracted.	,,	,,	All red; sometimes a black line in the cell-wall.
		—	Slight.	Sometimes broken and thickened.	Peculiarly contracted.	Benzopurpurin.	Victoria blue.	Cells red and blue in patches.
		Acid alcohol 24 hrs.	None.	Slightly thickened.	Contracted.	Crocein.	Methylene blue.	Cell-walls purplish red; protoplasm and nuclei blue.

TABLE VI *b* (*continued*).

Fixation and Staining of Vicia Faba (Root Apex) Embedded Material.

						Staining.		
Fixative.	*Time Fixing.*	*Treatment before Staining.*	*Cell Distortion.*	*Cell-wall.*	*Protoplasm.*	*For Cellulose.*	*For Pectin.*	*Staining Effects.*
Potassium ferrocyanide 2 %.	2 hrs.	Acid alcohol 24 hrs.	Slight.	Brittle.	Contracted in some parts.	Naphthol black.	Phenosafranin.	Red generally; nuclei black in some cases.
Ammonium sulphocyanide 4 %.	1½ hrs.	—	,,	Attenuated.	Contracted and precipitated.	,,	Fuchsin.	Whole section purplish, except root-cap, which was pink.
		—	,,	Slightly wavy.	Contracted.	Benzopurpurin	Victoria blue.	Cell-walls blue; nuclei red.
Calcium hypochlorite 4 %.	4 hrs.	—	Very slight.	Only slightly affected.	Sometimes contracted.	Naphthol black.	Phenosafranin.	Most cell-walls red, but a few black; nuclei black.
		—	Slight.	,,	Spread out in the cell.	Benzopurpurin.	Victoria blue.	Stained in patches red or blue; cell-walls sometimes red, sometimes blue; protoplasm sometimes blue.
Sodium hyposulphite 2 %.	2 hrs.	—	—	Little affected.	Very granular.	Naphthol black.	Phenosafranin.	Very heavy staining; staining was not 'clean'.
		—	Slight.	Attenuated.	Granular; not contracted.	Azo blue.	,,	Stained immediately very deeply; blue line where cells divide.

TABLE VI *b* (*continued*).

Fixation and Staining of Vicia Faba (Root Apex) Embedded Material.

Fixative.	*Time Fixing.*	*Treatment before Staining.*	*Cell Distortion.*	*Cell-wall.*	*Protoplasm.*	*Staining.*		*Staining Effects.*
						For Cellulose.	*For Pectin.*	
Hydroxylamine hydrochloride 1·8 %.	1½ hrs.	—	—	Little affected.	Contracted.	Azo blue.	Phenosafranin.	No blue except in the growing region, where there is a patch of cells all blue.
		—	—	”	”	Benzopurpurin.	Victoria blue.	Cell-walls blue; protoplasm and nuclei red.
Hydrochloric acid 1 %.	40 mins.	—	Marked.	Attenuated and broken down in many cases.	”	Naphthol black.	Phenosafranin.	Cell-walls red, in some cases traversed by a black line.
		—	”	”	”	”	Acid fuchsin.	Walls black; nuclei purple except a patch of cells just above root-cap, which are all purple.

TABLE VI *c*.

Fixation and Staining of Helianthus annuus (Root Apex) Embedded Material.

Fixative.	*Time Fixing.*	*Treatment before Staining.*	*Cell Distortion.*	*Cell-wall.*	*Protoplasm.*	*Staining.*		*Staining Effects.*
						For Cellulose.	*For Pectin.*	
Phenol 3 %.	4 hrs.	—	Marked.	Much broken in outer layers.	Disintegrated.	Crocein.	Victoria blue.	Cells all blue.
		—	”	”	”	Naphthol black.	Phenosafranin.	Cells all red.
Hydrocyanic acid 2 %.	5 hrs.	—	Very little.	Ribbon-like and sometimes broken.	Contracted.	Azo blue.	”	Stele red; outer layers of cortex blue.

TABLE VI *c* (*continued*).

Fixation and Staining of Helianthus annuus (Root Apex) Embedded Material.

						Staining.		
Fixative.	*Time Fixing.*	*Treatment before Staining.*	*Cell Distortion.*	*Cell-wall.*	*Protoplasm.*	*For Cellulose.*	*For Pectin.*	*Staining Effects.*
Hydrocyanic acid 2 %.	5 hrs.	—	Very little.	Ribbon-like and sometimes broken.	Contracted.	Naphthol black.	Phenosafranin.	All cells red.
Hydrocyanic acid ½ %.	4 hrs.	—	Slight.	Slightly affected.	Precipitated and spread through sections.	,,	,,	Very deep red, little black except in a few cell-walls.
		Acid alcohol 24 hrs.	Little.	Little altered.	Slightly contracted.	,,	,,	Only naphthol black showing; very heavy staining.
Ammonium sulphocyanide 2 %.	4 hrs.	—	—	Unaffected.	Contracted.	Benzopurpurin.	Victoria blue.	Mainly blue, but patchy.
		—	—	Ribbon-like.	Slightly contracted.	Crocein.	Methylene blue.	All blue.
Calcium hypochlorite 2·5 %.	4½ hrs.	—	—	Seldom broken.	Partially contracted.	,,	,,	Only crocein showing.
		—	A fair amount.	Ribbon-like; often broken.	Contracted and precipitated.	Azo blue.	Phenosafranin.	Fairly evenly blue and red; patch of red near root-cap.
Sodium hyposulphite 2 %.	4 hrs.	—	Slight.	Thickened and ribbon-like.	Granular and contracted.	Benzopurpurin.	Methylene blue.	Xylem and sclerenchyma deep blue; parenchyma purple.
Hydroxylamine hydrochloride 1·8 %.	4 hrs.	—	—	Little affected.	Contracted.	,,	Victoria blue.	Cell-walls blue; contents red.
		—	Somewhat distorted.	Often broken down.	Often contracted; sometimes disintegrated.	Naphthol black.	Phenosafranin.	All red; black lines in cell-walls and nuclei very dark.

TABLE VI*c* (*continued*).

Fixation and Staining of Helianthus annuus (Root Apex) Embedded Material.

Fixative.	*Time Fixing.*	*Treatment before Staining.*	*Cell Distortion.*	*Cell-wall.*	*Protoplasm.*	*Staining.*		*Staining Effects.*
						For Cellulose.	*For Pectin.*	
Hydrochloric acid 1 %.	16½ hrs.	—	Queerly distorted.	Often broken.	Precipitated as a solid mass.	Benzopurpurin.	Victoria blue.	Cell-wall blue in places; contents yellow.
		—	Marked.	Much broken in outer layers.	Disintegrated.	Azo blue.	Phenosafranin.	All very deep blue.
Sodium arsenate 2 %.	4 hrs.	—	Somewhat distorted.	Little affected; sometimes broken.	Often contracted and precipitated.	Benzopurpurin.	Methylene blue.	Patches of blue and red, cell-walls and contents.
		—	—	Unaffected in some parts; disappeared in others.	Slightly contracted.	Crocein.	,,	Blue; outer layers of root-cap red; nuclei dark blue.

TABLE VI*d*.

Fixation and Staining of Hyacinthus (Root Apex) Embedded Material.

Fixative.	*Time Fixing.*	*Treatment before Staining.*	*Cell Distortion.*	*Cell-wall.*	*Protoplasm.*	*Staining.*		*Staining Effects.*
						For Cellulose.	*For Pectin.*	
Phenol 5 %.	13 hrs.	—	Marked.	Broken up.	Contracted.	Benzopurpurin.	Victoria blue.	Uneven; mostly blue, little red showing.
		—	Slight.	Sometimes broken.	,,	Naphthol black.	Phenosafranin.	All red.
Lysol	13 hrs.	—	Marked.	Broken in many cases.	Granular and driven to sides of cell.	Azo blue.	,,	All purplish and obscured by protoplasm.
Hydrocyanic acid 5 %.	5 hrs.	—	—	Unbroken.	Contracted.	Naphthol black.	,	All reddish black.
		—	—	,,	,,	Benzopurpurin.	Victoria blue.	Cell-walls blue; nuclei, and often protoplasm, red.
Potassium ferrocyanide 5 %	4 hrs.	Acid alcohol 24 hrs.	Slight.	Some cell-walls broken up, others whole.	Much disintegrated.	Naphthol black.	Phenosafranin.	No double-staining; very dark.

TABLE VI *d* (*continued*).

Fixation and Staining of Hyacinthus (Root Apex) Embedded Material.

						Staining.		
Fixative.	*Time Fixing.*	*Treatment before Staining.*	*Cell Distortion.*	*Cell-wall.*	*Protoplasm.*	*For Cellulose.*	*For Pectin.*	*Staining Effects.*
Potassium ferrocyanide 5 %.	4 hrs.	—	Slight.	Some cell-walls broken.	Much disintegrated.	Azo blue.	Phenosafranin.	No double-staining.
		Acid alcohol 24 hrs.	,,	,,	,,	Crocein.	Methylene blue.	Nearly all blue, but some double-staining.
Ammonium sulphocyanide 2 %.	4¼ hrs.	—	Marked.	Broken in many cases.	Disintegrated, but not contracted.	Naphthol black.	Methyl violet.	All purple; nuclei very dark and swollen.
		—	,,	Often twisted and broken.	Disintegrated.	Benzopurpurin.	Victoria blue.	Walls and contents vary from red to blue.
Calcium hypochlorite 2·5 %.	2½ hrs.	—	—	Unbroken.	Slightly contracted.	,,	,,	Walls blue; contents red.
			Very marked.	Broken and thickened.	Contracted.	Crocein.	Methylene blue.	Indefinite.
		—	Slight.	Seldom broken.	Sometimes contracted.	Naphthol black.	Phenosafranin.	All red except a black line in cell-walls; nuclei almost black.
Hydroxylamine hydrochloride 1·8 %.	1¼ hrs.	—	Marked.	Broken and thickened.	Contracted.	,,	,,	All red.
		—	Decided.	Often broken.	,,	Crocein.	Methylene blue.	All blue.
Sodium arsenate 5 %.	13 hrs.	—	—	Unaffected.	,,	Azo blue.	Phenosafranin.	Stained badly with much precipitation of stains; nucleus red in centre and blue outside.
,,	13 hrs.	Acid alcohol 24 hrs.	—	,,	Peculiarly swollen.	,,	,,	Staining very pale; cell-walls blue, contents yellow.

TABLE VI *e.*

Fixation and Staining of Galtonia (Root Apex) Embedded Material.

						Staining.		
Fixative.	*Time Fixing.*	*Treatment before Staining.*	*Cell Distortion.*	*Cell-wall.*	*Protoplasm.*	*For Cellulose.*	*For Pectin.*	*Staining Effects.*
Phenol 2½ %.	2¾ hrs.	—	Slight	Often separated.	Not contracted.	Benzopurpurin.	Bismarck brown.	All brown except pericycle.
		—	,,	Cells separated, but cell-wall not broken down.	,,	Naphthol black.	Phenosafranin.	All red.
Phenol 1 % and acetic acid.	1 hr.	—	,,	Much broken in places.	Slightly contracted.	Azo blue.	,,	Cell-walls dark blue; contents red.
		—	,,	Badly broken.	,,	Naphthol black.	,,	No double-staining.
		Acid alcohol 24 hrs.	Marked.	Slightly broken in some cases.	,,	Benzopurpurin.	Methylene blue.	Walls bluish black; contents dark red; root-cap blue; part above root-cap often unstained.
		,,	,,	Badly broken in places.	Contracted in unbroken cells.	Crocein.	,,	Nearly all red; nuclei sometimes blue.
		,,	,,	Broken up.	Disintegrated.	Benzopurpurin.	Methyl violet.	All violet.
Phenol 3 %.	2¼ hrs.	—	,,	Often broken and peculiarly ‘lined’.	Contracted.	Naphthol black.	Phenosafranin.	All black, except for a red line in some cell-walls.
		—	,,	Completely broken up.	Disintegrated.	Benzopurpurin.	Methylene blue.	Very much over-stained blue.
Phenol 3 % and acetic acid.	2¾ hrs.	—	,,	Broken up	,,	Naphthol black.	Phenosafranin.	Too over-stained to see colour.
			,,	Completely broken up.	Contracted.	Benzopurpurin.	Methylene blue.	All red.

TABLE VI *e* (*continued*).

Fixation and Staining of Galtonia (Root Apex) Embedded Material.

						Staining.		
Fixative.	*Time Fixing.*	*Treatment before Staining.*	*Cell Distortion.*	*Cell-wall.*	*Protoplasm.*	*For Cellulose.*	*For Pectin.*	*Staining Effects.*
Hydrocyanic acid 0·5 %.	2 hrs. 50 mins.	—	Marked.	Broken down in many cases.	Not contracted.	Naphthol black.	Phenosafranin.	Cell-walls red; nuclei black.
		Acid alcohol 24 hrs.	”	”	Disintegrated.	”	”	All red.
Potassium cyanide 2 %.	3 hrs.	—	”	Broken and ribbon-like.	”	Azo blue.	..	Mainly blue; some patches of red.
Potassium ferrocyanide 5 %, and hydrochloric acid.	2 hrs.	Acid alcohol 24 hrs.	”	Broken in many cases.	”	Benzopurpurin.	Victoria blue.	All blue.
		—	”	Broken up.	”	Naphthol black.	Phenosafranin.	Mainly red; some patches of black.
Ammonium sulphocyanide 2 %.	3¼ hrs.	—	”	Often broken.	Contracted and often disintegrated.	Azo blue.	”	All red, except cuticle, which is blue.
		—	”	Quite broken up.	Disintegrated.	Crocein.	Victoria blue.	All red, except cuticle, which is bluish.
Calcium hypochlorite 2·5 %.	4½ hrs.	—	—	Little affected.	Much contracted.	Benzopurpurin.	”	Cell-walls and contents of root-cap blue; cuticle red; protoplasm bluish red.
Hydrochloric acid 1 %.	2¾ hrs.	Acid alcohol 24 hrs.	—	Cells sometimes separated.	Sometimes contracted.	Azo blue.	Phenosafranin.	All purple, except root-cap, which is red.
		—	Marked.	Cells often separated.	Contracted.	Crocein.	Methylene blue.	All blue; much overstained near tip.

TABLE VI *e* (*continued*).

Fixation and Staining of Galtonia (*Root Apex*) *Embedded Material.*

						Staining.		
Fixative.	*Time Fixing.*	*Treatment before Staining.*	*Cell Distortion.*	*Cell-wall.*	*Protoplasm.*	*For Cellulose.*	*For Pectin.*	*Staining Effects.*
Hydrogen peroxide.	2 hrs.	—	Marked.	Swollen and broken in many cases.	Disintegrated.	Crocein.	Methylene blue.	All pale brown.
			,,	Broken in many cases, but in red part it is unbroken.	,, except in red patch, where nucleus is visible.	Benzopurpurin.	,,	All blue; one part all red; here there is a blue line in the cell-walls.
Oxalic acid 5 %.	3 hrs.	—	,,	Disappeared.	Contracted.	Azo blue.	Phenosafranin.	All red.
		Acid alcohol 24 hrs.	,,	Broken in some cases.	Disintegrated.	Benzopurpurin.	Victoria blue.	Unevenly red and blue.

these two tables that the generalizations with reference to fixatives given in Table II (p. 280) are drawn.

The results obtained do much to confirm those of other investigators.

Allen (8) records that fresh material can sometimes be stained without the previous use of acid alcohol. That is the case when the 'cellulose' wall contains either more hydrocellulose or less oxycellulose than usual.

Mangin (13) observes that fresh material is difficult to work with, since good results can be obtained only when hydrocellulose is present, for oxycellulose is difficult to colour. In the same paper he states that cellulose is absent if no colour is produced after treatment with caustic alkali.

The extreme result of the action of caustic alkalis would be to remove a large part of the oxycellulose and hydrocellulose occurring in the cell-wall, though the solutions used in microchemical work would not be strong enough to do this completely. Accordingly the success obtained in staining cellulose from an alkaline bath may possibly be due to partial solution of these products, a certain proportion being retained by the unaltered cellulose, and reacting with the dye.

The results of this work may be briefly stated as follows:

(1) The 'cellulose' membrane consists of cellulose, oxycellulose, and hydrocellulose, the proportions of each constituent varying with the age and kind of cell.

(2) With fixed materials these proportions vary also with the kind of fixative, time of fixing, and concentration of the fixative.

An investigation into the nature of the cellulose membrane is not a simple matter. Certain facts have been ascertained; it remains to correlate their nature with the living processes of the plant.

SUMMARY.

1. The state of the cellulose, both natural or artificially produced, was studied with a view to determining whether oxidation or hydration had occurred.

The optimum conditions for distinguishing between cellulose and pectin after the use of a double stain are those causing minimum oxidation and maximum hydration without disintegration.

Tests for oxycellulose were performed on fresh and fixed material, and many fixatives were the subject of experiment. The results obtained with the stem and root of *Vicia Faba*, the stem of *Helianthus annuus*, and the root of *Hyacinthus*, are recorded in Table I, while Table II gives an account of the general effect produced by fixatives on cellulose and pectin in the plants studied.

2. Alcohol and glycerine proved objectionable reagents because of their obstinate retention by cellulose, and hence their liability to wash into the pectin, and so disturb its reaction to dyes.

3. A chemical classification (Tables III and IV) of the organic stains used in staining for cellulose and pectin shows that pectin is stained generally by organic compounds which are hydrochlorides of amines; cellulose by alkali salts of the disulphonic acids of compounds containing one or more azo-groups.

4. The effect of methylene blue proved uncertain in the experiments, and it is suggested that this is due to the presence of sulphur in the methylene blue molecule.

5. The results obtained by double-staining, after the use of fixatives, with freehand sections of the stem of *Helianthus annuus* and microtome sections of the root apex of *Vicia Faba*, *Helianthus annuus*, *Hyacinthus*, and *Galtonia*, are recorded in Tables V and VI.

The chemical state of the membrane probably takes the form of an equilibrium between cellulose proper, hydrocellulose, and oxycellulose. In most cases the two first mentioned predominate.

The investigation was carried out in the Botanical Department of Birkbeck College, University of London, and the author wishes to express

her thanks to Professor Dame Helen Gwynne-Vaughan for suggesting the research, for her valuable advice during its prosecution, and in particular for indicating the methods by which the use of alcohol and glycerine could be avoided.

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Evaporation in Wind.

A Criticism of the Contribution of H. Sierp and K. L. Noack to the Physics of Transpiration.

BY

WALTER STILES.

TWO years ago there appeared in one of the leading German botanical journals a paper which dealt with evaporation from water surfaces in moving air, a subject which is generally acknowledged to be of first importance in regard to the problems of transpiration from the plant. In view of this, and as the conclusions to which the authors of the paper come are so very divergent from those previously reached by English investigators, an examination of the results of these later investigations in relation to those obtained earlier in this country is called for. Moreover, as the problems of evaporation from the leaf, from the point of view of pure physics, are to a large extent the same as those of absorption of carbon dioxide by the leaf, it is essential that the physical laws of evaporation should be correctly formulated, not only because they are fundamental in regard to transpiration, but also because they have a direct bearing on the problems of assimilation of carbon dioxide by the leaf.

The authors of the paper in question, H. Sierp and K. L. Noack,[1] state that they undertook the investigation of the problem of evaporation in moving air because, as far as they knew, neither physicists nor botanists had attempted an exact treatment of the question. It is clear, therefore, that they were unaware that the problem had been investigated both from the botanical and physical point of view several years previously. This is the more surprising as the treatment of the subject from the botanical point of view by Miss Thomas and Ferguson appeared in one of the best known and most widely distributed botanical journals,[2] while the physical aspect of the

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[1] H. Sierp and K. L. Noack: Studien über die Physik der Transpiration. Jahrb. f. wiss. Bot., lx. 459-98, November 1921.

[2] Nesta Thomas and A. Ferguson: On the Reduction of Transpiration Observations. Ann. Bot., xxxi. 241-55, 1917.

[Annals of Botany, Vol. XXXVIII. No. CL. April, 1924.]

question was dealt with by the same authors in one of the best known physical journals,[1] and the very thorough consideration of the whole question by Jeffreys appeared in this same journal a little later.[2] The latter paper gave rise to discussion at the time,[3] while abstracts of the papers were published [4] and they were otherwise noticed.[5] Thomas and Ferguson did not emphasize the problem of evaporation in moving air, but results obtained under this condition were discussed, while Jeffreys dealt very deliberately with the problems of evaporation in moving air. That Sierp and Noack were unaware of this work is much to be regretted.

The method employed by Sierp and Noack is not above criticism. The evaporation measured was that from water contained in dishes placed in a vessel completely closed except for an entrance pipe and exit pipe through which the current of air passed. Now, under such conditions there seems no justification for supposing that the current of air passes with uniform velocity over the whole surface of the water; indeed, it seems extremely unlikely that this will be the case. When, therefore, the authors speak of a velocity of '4 litres an hour', that is, when 4 litres of air pass through the vessel in an hour, it seems almost certain that different parts of the water surface are exposed to air moving at different velocities, and the term '4 litres an hour' has no very exact significance. So that while on the whole a surface exposed to air moving with a velocity of '8 litres an hour' is subjected to the action of a stronger wind than one in which the air velocity is '4 litres an hour', it cannot be agreed that in one case the velocity of the air over the surface is twice that in the other. The method is, therefore, not likely to afford data of any value for obtaining the quantitative relations between evaporation and air velocity.[6] Also the translation of litres per hour into centimetres per second appears to have little quantitative value.

In calculating the effect of wind velocity on evaporation from their experimental results, Sierp and Noack start from a modification of Stefan's formula, $m = 4\,kr \,.\, \log \frac{P-p_2}{P-p_1}$, obtained by substituting a for $\log \frac{P-p_2}{P-p_1}$. The quantity a was then calculated for each of three wind velocities (4, 8, and 16 litres an hour) at three temperatures (15°, 25°, and 35°) from the formula $m = 4kra$, where m is the quantity of water evaporated in unit time, k is the coefficient of diffusion of water vapour, and r is the radius of the evaporating surface. They then found that a is not only a function of the temperature, but also of the dimensions of the evaporating surface, increasing with temperature and decreasing with increasing size of the surface.

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[1] Nesta Thomas and A. Ferguson: On Evaporation from a Circular Water Surface. Phil. Mag., xxxiv. 308–21, 1917.

[2] H. Jeffreys: Some Problems of Evaporation. Phil. Mag., xxxv. 270–80, 1918.

[3] Sir J. Larmor: Transpiration through Leaf Stomata. Phil. Mag., xxxv. 350–2, 1918. H. Jeffreys, with note by Sir J. Larmor: On Transpiration through Leaf Stomata. Phil. Mag., xxxv. 431–4, 1918.

[4] See Physiol. Abstr., vol. ii, p. 383, 1917; vol. iii, pp. 86, 148, 298, 1918.

[5] See R. C. Knight: Transpiration. Science Progress, xiii. 561–66, 1919.

[6] While this review was in preparation a notice of the paper by Sierp and Noack appeared (Nordhausen, in Zeitsch. f. Bot., xv. 575–6, 1923), in which much the same criticism is made in regard to method.

They further found that if a_x is the value of a when the velocity of the wind is x (measured in litres per hour) and a_{2x} when the velocity of the wind is twice this, the following relation holds, namely, $\log \frac{a_{2x}}{a_{2x} - a_x} =$ constant ($= \beta$), and that this constant is independent of the temperature, but decreases with increasing size of the water surface. This equation can be written in the form $a_{2x} = a_x \frac{10^\beta}{10^\beta - 1}$.

In the case of a dish of water 57 mm. in diameter at 25° the value actually found for β was 1·651. As the wind velocities actually employed in the experiments were very low, and much lower than those found in nature, Sierp and Noack calculated by extrapolation from this last equation the rates of evaporation in wind possessing velocities up to 32,768 litres per hour in order to obtain data for evaporation in air moving as rapidly as that in natural conditions. When this was done for two water surfaces of diameters 91·5 mm. and 57 mm. respectively, it was found that the ratio of water evaporated from the larger surface to that evaporated from the smaller surface in the same time at 25° with an air velocity of 32,768 litres an hour was 2·7 : 1, while the ratio of the areas of the surfaces was 2·6 : 1. The ratio actually found with a wind velocity of 16 litres an hour was only 1·2 : 1. The ratio of the diameters was 1·6 : 1. From these considerations the authors concluded that whereas with small wind velocities the rate of evaporation is roughly proportional to the diameter of the water surface, as the wind velocity increases the rate of evaporation becomes proportional to the area of the surface. That is, if we denote the rate of evaporation by e and the radius of the water surface by a,

$$e = ka^2,$$

where k is a constant.

Now Thomas and Ferguson by actual experimental observations, and Jeffreys from theoretical considerations, found for water surfaces of medium dimensions[1] that the actual relation of the rate of evaporation to the dimensions of the surface is very nearly expressed by the equation

$$e = ka^{1\cdot5}.$$

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[1] The term 'medium dimensions' is vague, and so a more exact description of the range of areas over which the relation here given holds is desirable. According to Jeffreys, when the wind velocity is 4 cm. per second a must lie between about 1 cm. and 25 metres; with a wind velocity of 400 cm. per second a must lie between about 10 cm. and 250 metres.

A possible explanation of this divergence between the results of actual experiments and of mathematical calculation on the one hand, and those obtained by extrapolation on the other, at once suggests itself. The formula used by Sierp and Noack becomes in general

$$a_{2^n x} = a_x \left\{ \frac{10^\beta}{10^\beta - 1} \right\}^n .$$

Now in extrapolating from wind velocities of 4 and 8 litres an hour to one of 32,768 litres (= 2^{15} litres), any significant error in $\frac{10^\beta}{10^\beta - 1}$ will be tremendously magnified in the value for $a_{2^n x}$. It is obviously necessary to examine the magnitude of the error that may possibly occur in the value of a for high velocities found by extrapolation.

Fortunately Sierp and Noack provide data which enable this to be done. Thus, for the constant β they found 6 values for each of the water surfaces they used. These values enable the probable error to be calculated. When this is done the values for β along with the probable errors for the three water surfaces they employed are found to be as follows:

Diameter of Water Surface in mm.	*Mean Value of β.*	*Probable Error of Mean Value of β.*
37	0·490	±0·020
57	0·404	±0·010
91·5	0·361	±0·005

Although the number of observations is small, it is sufficient to give an idea of the magnitude of the error possible. If it is assumed that the actual error in β is no more than ±0·010, certainly a very moderate estimate, calculation shows that the error in $a\,2^{15}$ is of the order ±20 per cent. Now, if such an error in the positive direction existed in the value of a found for the surface of 57 mm. diameter and one in the negative direction in the case of the larger water surface, the ratio of the quantities evaporated would be in the proportion, not of 2·7 : 1, but of about 1·75 : 1. Now the ratio of the diameters of the surfaces is 1·6 : 1, of $(\text{diameters})^{1 \cdot 5}$ 2·03 : 1, and of the areas 2·6 : 1. It therefore seems clear that the possible error is so great in the extrapolated values found by Sierp and Noack that they give no exact information with regard to the relation between evaporation and dimensions of the water surface when a strong wind is blowing. Having regard to the error involved, their results are quite consistent with the law previously found by Thomas and Ferguson, and by Jeffreys, to the effect that the rate of evaporation is approximately proportional to $a^{1 \cdot 5}$, where a is the radius of the surface. There is, therefore, no reason to suppose that as a result of Sierp and Noack's work the conclusions of Thomas and Ferguson and of Jeffreys are other than correct.

Sierp and Noack also discuss the evaporation of water through multiperforate septa. If I follow their argument correctly, they appear to misunderstand the conclusions of Brown and Escombe on this subject. Thus they give data with regard to three multiperforate septa, each of area 25 sq. cm., in which, the pores being 10 diameters or more apart, there is no interference in evaporation through neighbouring pores. The data are as follows:

	Septum 1.	*Septum* 2.	*Septum* 3.
Number of pores	25	625	15625
Diameter of pore	1 mm.	0·1 mm.	0·01 mm.
Area of pore	0·785 sq. mm.	0·0079 sq. mm.	0·000079 sq. mm.
Total area of pores	19·63 ,, ,,	4·94 ,, ,,	1·23 sq. mm.
Diameter of circle with this area	5 mm.	2·51 mm.	1·25 mm.

They then say that a comparison of the diameters of the total areas of the pores, which diameters, according to Brown and Escombe, determine the magnitude of the evaporation, shows that this, in spite of the enormous increase in the number of pores, regularly decreases, so that under the conditions required by Brown and Escombe for no interference between evaporation from different pores, it can never be the case that reduction in the size of the pores can bring the diffusion up to that which would take place from a corresponding water surface, and that Jost and Renner are in error in stating that it can.[1]

Now Brown and Escombe said nothing of the kind. According to them the evaporation is not proportional to the diameter of an imaginary circle having the area of the total surface of the pores, but to the sum of the diameters of the pores, for there is supposed to be no interference between the evaporation through neighbouring pores, and the evaporation through each pore is proportional to the diameter. The evaporation through the three septa, therefore, would, according to Brown and Escombe, be in the proportions 5 : 12·5 : 31·25, not as 5 : 2·5 : 1·25. Thus, reduction in the size of the pores, provided the distance between the pores relative to the diameter of a pore remains the same,[2] can bring the diffusion up to the amount which would occur from a water surface of the same dimensions as the septum. Jost and Renner are thus correct when they say that diffusion or evaporation through a multiperforate septum may be the same as if there were free diffusion from the surface with no interfering septum.

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[1] 'Ein Vergleich der Durchmessergrösse der Gesamtfläche, die nach Brown und Escombe für die Grösse der Evaporation ausschlaggebend ist, zeigt, dass diese trotz des enormen Anwachsens der Lochzahl stetig abnimmt. Es ist also nicht richtig, wenn Jost bei der Besprechung der Wirkung multiperforater Septa auf die Diffusion sagt, dass die Diffusion "im Extremen einen solchen Betrag erreichen kann, wie wenn die Haut ganz fehle, wie wenn eine völlig freie Diffusion stattfände", eine Ansicht, die auch Renner im Handwörterbuch der Naturwissenschaften vertritt. Wenn also die einzelnen Löcher so weit auseinander liegen, wie Brown und Escombe es verlangen, so dass sie sich gegenseitig nicht beeinflussen, so muss trotz der gesteigerten Diffusionsgeschwindigkeit der absolute Wert der Diffusion bei Abnahme der Lochgrösse stetig kleiner werden. Es kann unter diesen Bedingungen nie der Fall eintreten, dass durch fortgesetzte Verkleinerung der Löcher die Diffusion den Betrag der der Gesamtplatte entsprechenden Wasserfläche annimmt' (Sierp and Noack, pp. 483–4).

[2] It will be observed that in the case of the three septa described by Sierp and Noack the pores are 10, 20, and 40 diameters apart in septa 1, 2, and 3 respectively. The reasons which led Sierp and Noack to select these particular septa to illustrate their discussion are obscure.

As a matter of fact Jeffreys found that the evaporation from the surface of a leaf in still air is likely to be the same as that from a wet surface of the same size and shape until the diameters of the stomata are reduced, in some cases, to one-fiftieth of that of the fully open stomata. The actual degree of closure necessary before each stoma acts independently of the others will depend on the size of the stomata and their distribution over the surface of the leaf. Under ordinary conditions, however, the 'wet leaf' law will hold. This being the case, the evaporation will follow the ordinary law of evaporation from water surfaces, and therefore, as Jeffreys has shown, it will, within limits, be proportional to $a^{1 \cdot 5}$ when a steady wind is blowing over the surface, where a is proportional to the linear dimensions of the leaf. The complications arising when the stomata are sunk below the surface of the epidermis need not be discussed here. The few experiments on evaporation through multiperforate septa performed by Sierp and Noack are scarcely sufficient to enable any definite conclusions to be drawn, but they appear quite consistent with the 'wet leaf' law and the relation $e = ka^{1 \cdot 5}$, and the expression of a different opinion by Sierp and Noack need not therefore detain us further.

SUMMARY.

A critical consideration of the work of Sierp and Noack shows that the results obtained by these authors are not inconsistent with the relation found by Thomas and Ferguson, and by Jeffreys, that the rate of evaporation from a free water surface of medium dimensions exposed to a wind is proportional to $a^{1 \cdot 5}$, where a is proportional to the linear dimensions of the surface. The conclusion of Sierp and Noack that the evaporation is proportional to a^2 can be explained partly, perhaps, on account of their experimental arrangement, which is not above criticism, and certainly on account of the error involved in their calculation of the rates of evaporation in air moving at high velocities.

The results of Sierp and Noack with multiperforate septa are not inconsistent with the laws governing the evaporation of water through such septa exposed to moving air previously propounded by Jeffreys.

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The Response of Plants in Soil- and in Water-culture to Aeration of the Roots.

BY

R. C. KNIGHT, D.Sc.,

Department of Plant Physiology and Pathology, Imperial College of Science and Technology, London.

With one Figure in the Text.

THE influence of thorough cultivation of the soil upon crop production is a fact too well established to need elaboration, but the same can hardly be said of the explanation of this effect. The beneficial results are variously attributed to the conservation of soil moisture, to soil aeration, and to increased fertility due to weathering, among other factors. It is probable, as is usual in such cases, that the effect is due in varying degree to many factors rather than to a single one, and moreover local conditions doubtless play a considerable part in determining which factors are most important. Investigation of some of these factors is proceeding and attention has been directed to soil aeration. Experiments have been carried out at Cheshunt (1), in which the soil in tomato-houses was artificially aerated by underground pipes. Air was forced into the soil during two 15-minute periods per day, with the result that a 10 per cent. increase in yield was obtained. In addition, earlier ripening was observed in the fruit on the aerated plants, and a higher proportion of first-grade fruit was produced. The aerated plants were healthier and less attacked by 'mildew', and the foliage was better in quality than in the controls.

This is only a single observation, but it is the only work on the same scale known to the writer. Noyes (16) has dealt with the problem of the cultivation of soil and its effect upon crop production, and attaches most importance to the indirect influence of aeration. This work is further dealt with later.

It is evident that, in order to investigate the reaction of plants to soil aeration, it is first necessary to obtain an understanding of the physiological processes of the root. The progress which has been made in the investigation of the physiology of the root system of the plant is very

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slight when compared with the knowledge which we possess with reference to the rest of the plants. The reason is not far to seek. It is not that the importance of the root system is not recognized, but rather that the difficulties of manipulation are much more formidable when working with roots than in investigations of aerial parts. The presence of such a complex medium as the soil is always a disturbing factor, unless the plants are grown, for example, in water-culture, and under these circumstances there may always be urged the objection that the behaviour of a plant in water-culture is not necessarily a criterion of what its reactions would be under normal conditions of growth. It is doubtless due to such considerations as these that our knowledge of root physiology is so meagre. However, even a slight knowledge of the physiology of the root has led to the recognition of the importance of the nature of the soil gases and of their relation to the respiratory activities of roots.

Russell and Appleyard (16) carried out many analyses of soil air, and found that in the upper layers of soil the composition was very similar to that of atmospheric air—the difference being a small increase of CO_2 at the expense of oxygen. The fluctuations in composition were found to be considerable, and were attributed to biochemical changes. Many series of experiments have been carried out to investigate the influence upon plants of changes in the composition of the atmosphere surrounding the roots. Noyes (13) found that if the soil atmosphere were replaced by CO_2, rapid deterioration and death of the plants followed. He also observed that maize was less sensitive than tomatoes towards CO_2. This difference in behaviour of different species has also been recorded by other workers. Noyes, Frost, and Yoder (15) worked with pot-cultures of various plants, the roots of which were subjected to the action of a stream of CO_2. Again it was found that the effect varied very much with the species used. *Phaseolus*, for example, proved to be almost unaffected by the CO_2, but with other species the usual result was a decrease in the amount of root-formation.

In the majority of investigations of the influence of different soil atmosperes upon roots, attention has been directed either to increasing the air-supply or to raising the CO_2 concentration. This of course is the necessary preliminary step, but no analysis of the problem can be considered complete unless distinction is made between the individual effects of oxygen and carbon dioxide. Roots of buckwheat in an atmosphere of nitrogen were observed by Free (8) to be quite normal. It might perhaps have been expected that the absence of external oxygen would restrict growth, but, as will be noted later, the behaviour of buckwheat towards changes of oxygen content in the medium surrounding the roots is rather unusual. On the other hand, Free found that if pure CO_2 were supplied to roots of buckwheat, death resulted very soon. These experiments show very clearly the distinction between the merely negative effect of lack of oxygen and the positively toxic effects of CO_2. Thus an experiment in which CO_2 concentration in the soil is increased, or alternatively, one in which CO_2 concentration is reduced to a minimum by a continuous supply of CO_2-free air, cannot alone be of much assistance in the analysis of the problem.

At present our knowledge does not extend far beyond the elementary stage indicated above, but data are being accumulated with reference to the response of different species to 'aeration', i.e. reduction of CO_2 concentration, and to CO_2 treatment, i.e. increase of CO_2 concentration and accompanying decrease of oxygen and nitrogen.

Livingston and Free (10) worked with several species, and found that in most plants the replacement of soil oxygen by nitrogen quickly resulted in a falling off in the rate of water absorption by roots, and was soon followed by death. A notable exception was found in the case of *Salix nigra*, a swamp willow, which was capable of enduring almost complete exclusion of oxygen from the roots. In view of the nature of the habitat of this species it is interesting to find this peculiarity, and the authors suggest that in this case root respiration may be normally carried out at the expense of internal oxygen, since in the water-logged soil oxygen supply must be practically nil. In this connexion some experiments recorded by Hole (9) are interesting.

He found that in water-culture water alone is not harmful to roots, and in sand-cultures the plant is not injured if the sand is water-logged, provided the water is kept in motion. The water in motion presumably removes from the roots the CO_2 produced by respiration and maintains a constant, if small, supply of oxygen. A concentration of 0·5 grm. CO_2 per litre in the culture solution was, however, sufficient to kill roots growing in water. Bergman (3) found that submerged roots do not produce root-hairs. He also records the death of the plants following the replacement of soil air by CO_2. Land plants lose their turgidity if the soil is water-logged, and the transpiration rate decreases. These facts conform with the observation of Livingston and Free (above) that the rate of water absorption decreases when oxygen is not available. Bergman attributes the capacity of marsh plants to grow as they do with roots submerged, to the aerenchyma system which is present, an hypothesis which is also consonant with that held by Livingston and Free. Norris (12), in experiments on *Zea Mais*, showed that the root cortex will develop an aerating system if the oxygen supply is poor. Cannon and Free (5) found a retardation of root growth following the introduction of CO_2 into the atmosphere bathing the roots, and the amount of retardation increased with the concentration of CO_2. Specific differences in response were again noticed. *Prosopis velutina* would still grow to a small extent with its roots in an atmosphere of 50–75 per cent. CO_2, whilst the growth of *Opuntia versicolor* was totally inhibited by this concentration. Artificial reduction of the CO_2 content of the soil atmosphere by a continuous supply of air generally favoured growth. The work on *Prosopis* and *Opuntia* was extended by Cannon (4), who found that not only is *Prosopis* less sensitive than *Opuntia* to high CO_2 concentration, but it is also less sensitive to low oxygen concentrations.

Andrews and Beals (2) found that aeration of the solution very considerably increased the yield of maize grown in water-culture, and they also recorded the development of wide air-spaces in non-aerated plants, the latter observation confirming the results of Norris. Cerighelli (7) investigated the respiratory ratio of attached and of detached roots, and found it to vary from 0·7 to 1·0 according to species. He also observed that much CO_2 was retained in the root tissues. This being so, it is to be expected that artificial aeration, by reducing local concentrations of CO_2, will increase growth. Cannon and Free (6) record some observations on the capacity of plants to develop special root systems adapted to particular conditions. Sunflowers were found to produce a new root system when the soil atmosphere was replaced by nitrogen, and a similar result was obtained when plants were transferred from soil to culture solution. The present writer has also observed that a change in the composition of a culture solution sometimes results in the death of the old root system and the production of a new one. Cannon and Free observed that the roots formed in soil in the absence of oxygen and those formed in culture solution differed from normal roots in being short, thick, and devoid of root-hairs. The writers regard these facts as suggesting that the lack of root-hairs on certain swamp plants is due to deficiency of oxygen rather than to surplus of water. Cannon and Free also found that *Zea Mais*, if deprived of soil oxygen, ceased growth for a time and then recommenced growth at a slower rate. This is regarded as evidence of a physiological adjustment to anaerobic conditions. Evidence has already been adduced to show that this plant may become morphologically modified as the result of bad aeration (2, 12).

Stiles and Jørgensen (18) investigated the effect of aeration on plants growing in water-culture. Air was bubbled through the culture solution, and in the case of barley and balsam increased yields were obtained in the aerated series. Buckwheat, on the other hand, produced no increase in crop as the result of aeration, a result similar to that obtained by Free (8).

From the foregoing summary of published work it will be seen that on the chief aspects of the question of root aeration there is extraordinary unanimity. Workers are agreed that species differ in their reaction to removal of CO_2 from the soil by aeration, but the general result is an increased yield. It is also agreed that any effect on the plant resulting from poor 'aeration' may be the result of two factors, excess of CO_2 and lack of oxygen, and it is realized that these two factors must be dealt with separately in analysing the question of soil aeration.

It should be pointed out here that the question has recently been raised whether the beneficial influences on crop weight, which have been shown to result from soil aeration, are really due to the direct effect upon root activity of changes of CO_2 concentration and of oxygen supply. Lyon (11) records a series of field and laboratory experiments on the tillage of soil and its nitrogen content, and concludes that the quantity of nitrate in the soil is dependent to a large extent on the efficiency of aeration. It appears from this that the effect of good aeration on a crop may be indirect, through the increased activity of soil organisms. Noyes (14), working on the problem of water requirement, reached a conclusion which also has a bearing on the present problem. Noyes found that in well-cultivated plots the water requirement of the crop, i.e. the weight of water used in the production of unit quantity of dry matter, was less than in badly cultivated or uncultivated areas. He does not associate the importance of cultivation with water conservation, as has commonly been done, but rather with the increase of bacterial action following upon better soil aeration. The point of view set forth in these two papers clearly points the way to still further analysis of the question of soil aeration.

In view of the important practical results which have been obtained in aeration experiments, it was decided to undertake an investigation of the question of aeration, and a series of experiments was accordingly devised. Circumstances have prevented the continuation of the work beyond the preliminary stages, but it is considered desirable to record the results obtained to supplement the groundwork already laid out by other workers, in preference to withholding them until opportunity shall arise to resume the work.

Aeration experiments have been carried out using plants growing in soil- and in water-culture, and the methods employed have not yet proceeded beyond the primary stages. The treatment of the plants has been confined to removal of CO_2 from the soil (or culture solution) by a stream of CO_2-free air, and, at the other extreme, to the prevention of the escape of CO_2 from the soil. No attempt has yet been made to discriminate between the two factors referred to above, the early work having been directed to finding plants which were sensitive to aeration.

I. *Pot-culture Experiments.*

Experiment 1. In this experiment maize (Barr's Golden Bantam) was grown in soil in 10 in. glazed earthenware pots in a cool greenhouse. Broken crocks were placed in each pot to a depth of one inch, and these were overlaid with a piece of coarse wire gauze in order to ensure uniform distribution of air in the aerated series. Each pot contained twenty-eight pounds of soil and five plants, and was fitted with two tubes for sampling the soil atmosphere, one extending to within an inch of the bottom of the soil and the other an inch below the upper surface. Water was added through a wide tube terminating in a 'pocket' of broken crocks in the middle of the pot. The soil used was a light garden loam, the character of which is indicated by the following constants:

The minimum water content determined by the method described by Hilgard was 17·2 per cent., and the maximum water content, estimated by means of Hilgard's soil-water vessels, was 57·6 per cent., these figures being the averages of seven determinations. The pore space of the soil was found by the simple percolation method. Air-dry soil, i. e. 1 to 2 per cent. water, was packed into a cylinder and water was allowed to percolate through from below. The maximum quantity of water which could thus be introduced was 50·4 per cent. by volume, but this figure was obviously not an accurate expression of the amount of air-space in the soil, on account of the impossibility of replacing all air-bubbles by water, however slow the percolation. Accordingly the experiments were repeated using an evacuation method. A cylinder, A, was connected with a manometer and an air-pump, and a similar cylinder, B, was connected with A. A was evacuated to as low a pressure as possible and the manometer reading was taken. The stopcock connecting A with B was then opened and the manometer was read again. B was then filled to the stopper with soil. A was evacuated to the same pressure as formerly, the connecting stopcock again opened and the manometer read. From the three manometer readings thus obtained, the percentage air-space in the soil may be readily calculated.[1]

The use of such methods has been deprecated on the ground that the physical characters of the soil are radically altered by such extreme pressure changes, an objection which, however, applies similarly to the percolation method.

By the evacuation method the air-pore space in the air-dry soil was found in successive determinations to be 58·2, 58·6, 58·6, 58·5, 57·8, and 57·0—average 58·1 per cent. These figures are in close agreement, when it is considered that all errors and irregularities of packing the soil are included. As might have been expected, the air-pore space is higher than the water-percolation method indicated. Similar trials, carried out with the soil containing 15 per cent. water by weight, determined air-pore space as 43 per cent. In view of the contraction which occurs on wetting an air-dry soil it is to be expected that the sum of percentage water content and percentage air-pore space will decrease with increasing water content. (The specific gravity of the air-dried soil-air system was about 0·9.)

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[1] If pA is the manometer reading when A is evacuated,
pB is the manometer reading when the connecting stopcock is open—B containing air, and
pC is the manometer reading when the connecting stopcock is open—B containing soil,
then the percentage of air-space in the soil by volume is

$$100 \times \frac{pB\ (pA - pC)}{pC\ (pA - pB)}$$

The seeds used were graded, and only those weighing more than 0·29 grm. and less than 0·32 grm. were used. About 30 per cent. of the sample fell within these limits. The seeds were soaked in water for twelve hours and germinated on sand before planting in order to secure uniformity in the plants. On March 20 the germinated seeds were planted and the water content of the soil brought to 25 per cent. It was maintained at this level for ten days, by which time the plants were established. After this date the water content was made up to 23 per cent. at each watering. All pots were watered through their central tubes at intervals determined by external conditions. Water was added to compensate for loss by surface evaporation and transpiration, and the water content of the soil was not allowed to fall more than 1½ to 2 per cent. before replenishing. No allowance was made for the water present in the growing plants, since it was calculated that at the time of harvesting the reduction of soil water content as the result of neglect of this factor would be less than 1 per cent. At each watering the position of the pots was changed in regular rotation in order to reduce as far as possible differences due to local conditions. On March 30, ten days after sowing, wax seals were applied to twelve pots of the series and aeration was commenced in another set of twelve. A third set was maintained under ordinary conditions as a control series. The wax seals were composed of three and a half parts of paraffin (m.p. 45° C.) and one part of vaseline. The mixture was applied in a liquid state as cool as practicable, the stems of the plants being protected by paper collars. After solidification of the seal, the collars were removed and the final seal around the plants was made with a softer mixture of paraffin and vaseline in equal proportions. The sampling tubes were sealed, and the watering tube was corked. It was found to be difficult to ensure a complete seal, the wax tending to break away from the pot at some point, and frequent repair of these fractures was necessary. Also small leaks of air were almost always to be detected between the stems and the encircling leaves of the maize plants. These latter were unavoidable when using maize, but they were probably also relatively small. Thus the series of pots which were 'sealed' could only be regarded as partially sealed. The efficiency of the seals is revealed by the soil gas analyses recorded later, and by comparison of these results with those obtained for a later series of cultures, which, partly on account of the difficulty of sealing, were carried out with narrow-necked vessels.

Aeration of the second series was carried out through a tube passing into the layer of broken crocks below the wire gauze in each pot. Before entering the pot the air was led through a small wash-bottle which served as a means of rendering the air moist, thus reducing its capacity for removing water from the soil, and also as a means of determining the rate of the air-stream for purposes of control. A screw clip for each pot controlled the speed of the air-stream. Each pot was supplied from a side tube connected with the main air-supply, all the pots being thus in parallel. The pressure required to force air through the system was first supplied from a motor-driven pump and later from a large gasholder. The latter needed frequent attention for refilling, and, moreover, the pressure developed fell continuously as the reservoir was being emptied of air. Eventually the air pressure was supplied from the water-tap by means of a simple apparatus which has proved very satisfactory. It consists of a wide-mouthed bottle, *a*, fitted as shown in the diagram (Fig. 1). The tube *b* is attached to the water-supply, and as water runs in air is forced out through tube *c*, which is connected with the main air-supply to the pots. Water rises in *d* and *e* to a height depending upon the pressure against which the apparatus is working. The level in *e* remains constant, but the level in *d* rises with the level in *a*. Thus air is forced through *c* until the level in *d* reaches the top of the siphon tube, through which the water from *a* now begins to escape. Air to replace it is automatically drawn in through *e*, and at the same time in *c* a small backward pull is set up, the magnitude of which is determined mainly by the depth of water in *e*. To prevent a backward flow through *c* a wash-bottle is inserted to act as a valve, if the system does not already include one. When the water level in *a* reaches the bottom of *d*, the siphon 'breaks', and by the inflow of water through *b* the pressure rises and air again begins to pass through *c*. Thus an almost continuous flow of air is maintained. The critical portion of the apparatus is the siphon, which must 'make' and 'break' sharply. To facilitate the 'break' a wide end is blown on the short leg, and to ensure a good 'make' the inside of the siphon tube from *g* to *h* is coated with paraffin wax of low melting-point, which may need renewal after a week or two. If the whole of the siphon tube is waxed it is found that the 'break' is not always sharp, and chains of large bubbles persist. In order to utilize the full capacity of the reservoir the length of the short leg of the siphon tube is so chosen that the water level inside it reaches the top when the bottle is full. The internal diameter of the siphon tube is approximately ¼ in., but this, as well as the size of the reservoir,

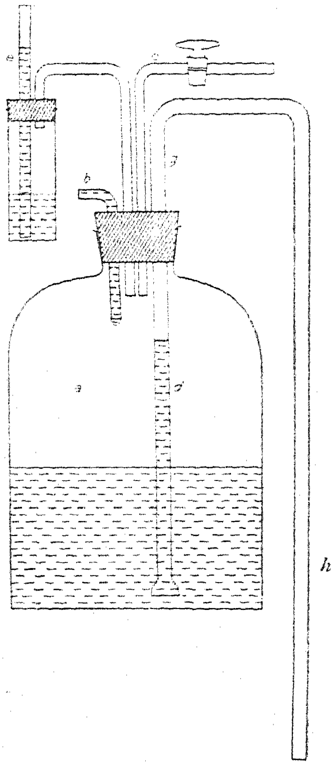

FIG. 1.

must naturally be adjusted to the conditions of the experiment. Although no failure of the apparatus has occurred, it has always been deemed advisable to arrange that tube *c* is above the other tubes, so that, in case of accident, water would not be forced through this air exit. The air-stream produced by this apparatus is practically constant after the tap has been running for a short time, but it is not continuous. There is a short interval during which no air passes, each time the reservoir is emptied, but in aerating soil- and water-cultures this periodical cessation of the air-supply cannot be of importance.

During the earlier part of the growing period an attempt was made to obtain indications of the rate of growth of the various cultures. Measurements were made at intervals of the length of each plant, from the soil to the tip of the highest leaf when the plant was held upright. Leaf counts were also taken for each plant. Owing to the time occupied by other operations concerning these and other cultures, these measurements were not continued throughout the life of the plants. The results obtained give curves which are almost coincident in all three series.

There is no indication of sigmoid form in any of the curves, and this may be explained by the fact that the period which they represent corresponds with the stage of most rapid growth and does not include either the earlier or the later stages of slower growth. From the figures available it is not possible to say whether increase in length or in leaf number gives a very close approximation to growth-rate in maize, although for barley Stiles and Jørgensen (18) found leaf number and dry weight results to be in good agreement, and the leaf number figures fall on the first portion of a sigmoid curve.

On May 21 and 22, analyses of the soil gases were made by means of a Haldane apparatus, and the plants were harvested on May 23, roots and shoots being collected separately. The soil was washed out from the roots, a process which was fairly easy, if laborious, with the light soil used.

TABLE I.

Analysis of Soil Atmosphere in Pot-culture Experiments previous to Harvesting.

Series.	*Bottom.*		*Top.*	
	O_2	CO_2	O_2	CO_2
Aerated	21·3	0·22 ± 0·02	—	—
Sealed	19·5	1·80 ± 0·04	19·5	1·30
Control	21·1	0·33 ± 0·03	21·0	0·08

The results of the soil-gas analyses are shown in Table I. Duplicate samples were taken from near the bottom of each pot, so that the figures shown represent the average of twenty-four analyses. Samples were also taken from near the top of each pot, but the analyses obtained were considered unreliable owing to the fact that as a sample is extracted, outside air replaces it and probably becomes mixed with the sample.

The individual analyses varied considerably, from 0·07 per cent. CO_2 to 0·33 per cent. CO_2 in the aerated series, from 1·52 per cent. CO_2 to 2·07 per cent. CO_2 in the sealed series, and from 0·17 per cent. CO_2 to 0·55 per cent. CO_2 in the control series.

The difference between the series is small, but apparently significant. In the aerated series the formation of CO_2 in the soil was sufficiently rapid to produce an accumulation in the soil in spite of the stream of fresh air passing through. It is evident that the speed of this stream might have been increased with advantage, in order further to reduce the CO_2 concentration. The concentration of CO_2 in the control pots was lower than was expected, and obviously in the light soil used the escape by diffusion was rapid. In the sealed pots CO_2 had accumulated at the expense of some oxygen, but it seems likely that some CO_2 had been lost by leakage owing to the difficulty of effecting a complete seal. The dry weights of the crop are given in Table II.

TABLE II.

Average Dry Weight per Plant in grm. of Maize Cultures under different Conditions of Soil Aeration.

Series.	*Shoots.*	*Roots.*	*Whole Plants.*
Aerated	2·64 ± 0·08	0·25 ± 0·01	2·89 ± 0·08
Sealed	2·05 ± 0·04	0·15 ± 0·002	2·20 ± 0·04
Control	2·00 ± 0·05	0·17 ± 0·01	2·17 ± 0·06

The aerated series shows an increase of over 30 per cent. on the control series. The increase is shown in both shoots and roots. The sealed series, on the other hand, gave the same result as the normal series, within the limits of experimental error. It is not easy to correlate this result with the figures obtained for CO_2 content of the soil (Table I). At first sight it would be expected that the similarity between the soil atmospheres of the aerated and the normal, and also the high CO_2 content of the soil in the sealed series, should be reflected in the crop weights, yet the results do not bear this out. In the attempt to account for the discrepancy suspicion fell on the CO_2 analyses for the aerated series. Aeration had been suspended on the morning the analyses were made and the pots were dealt with in succession, so that by the time the last pot was sampled it had received no air for some hours. It appeared possible that in this interval CO_2 had accumulated in the soil in sufficient quantity to invalidate the analyses, and a study of the individual results confirmed this. The samples from the first five pots gave an average CO_2 content of 0·11 per cent., and the last five 0·31 per cent., showing that the figure 0·22 per cent. CO_2 is not really representative of the soil atmosphere conditions during aeration. The correct figure is probably nearer 0·07 per cent.—the lowest of the series. Further confirmation was obtained later, when working on the accumulation of CO_2. A sealed pot was set up in the usual way, but without any plants, and air was passed through rapidly for one hour. Aeration was then stopped and within a few minutes a sample of soil atmosphere was removed for analysis. Similar samples were taken at hourly intervals for a time and then at intervals of some days. The results are given in Table III.

TABLE III.

Accumulation of CO_2 in Soil Atmosphere of a Loam.

Time.	*% CO_2 by Volume.*
11.5.'21.	
[10.30–11.30 a.m., soil aerated.]	
11.40 a.m.	0·10
12.40 p.m.	0·55
1.40 p.m.	0·98
3.0 p.m.	1·30
3.40 p.m.	1·52
4.40 p.m.	1·77
12.5.'21.	
11.40 a.m.	6.60
13.5.'21.	
12.20 p.m.	11·19
18.5.'21	16·9
30.5.'21	30·6
3.6.'21	34·0

Oxygen content 3.6.'21, 0·5 %.

The rapid accumulation of CO_2 in a sealed pot is very striking, and although the soil in this case was heavier than in the cultures under discussion, there appears to be little doubt that the high CO_2 figure (0·22 per cent.) obtained in the aerated series was due to accumulation after the cessation of aeration, and that the CO_2 content during the growth period of the plants was less than 0·1 per cent.

From consideration of Tables I and II, in the light of this conclusion, it appears that maize is sensitive to changes of concentration of CO_2 in the soil atmosphere, especially when the concentration is very low. A change from 1·8 per cent. to 0·3 per cent. appears to have had no effect, but the removal of the greater part of the last 0·3 per cent. resulted in a considerable increase in dry weight.

At the close of this experiment some reserve cultures which had not been included were subjected to treatment with atmospheres containing high concentrations of CO_2 (since 1·8 per cent. appeared to have no effect) in order to obtain information as to the rate of escape by diffusion and also the time required for a definite effect to become visible. Cultures, the soil of which had been treated with 5 per cent., 10 per cent., and 15 per cent. CO_2 respectively, continuously for five days, showed no ill effects at the end of that period. A stream of pure CO_2 produced wilting in less than two days and the soil atmosphere contained 53 per cent. to 65 per cent. CO_2 at different times during treatment. Five hours' treatment of a normal pot with 24 per cent. CO_2 resulted in raising the CO_2 content of the soil gas to 6 per cent., but in a wax-sealed pot the CO_2 content was increased to 12 per cent., and in the latter case the plants showed signs of wilting in two days. These observations show that the loss of CO_2 from an open pot is rapid, and there is evidence that relatively high concentrations of CO_2 are required to kill maize.

Experiment 2.[1] Giant Caragua maize was used in this case and the plants were grown one to each 1,200 c.c. glass milk-bottle. The narrow necks of these bottles made the sealing much easier than in the case of the 10 in. pots. The bottles were covered with black paper, and the general procedure was very similar to that in the previous year's experiment.

The soil used was a fairly heavy fibrous loam with a minimum water-holding capacity of 27·6 per cent. and a maximum of 69·5 per cent. The pore-space of the soil was 39·4 per cent. by volume when the soil moisture was 32 per cent. and 59·2 per cent. by volume when the soil moisture was 13·8 per cent. The seeds were graded by weight and only those weighing between 0·34 grm. and 0·43 grm. were used. The seeds were sown on March 16, 1921, and the soil moisture content was fixed at 30 per cent. When the plants were well established aeration was commenced in one series and a second series was sealed with the wax mixture. Watering was carried out precisely as before at intervals, and analyses of the soil atmosphere were made from time to time. On April 26 the soil gas in the sealed series contained on the average 11·6 per cent. CO_2 and 9·5 per cent. oxygen. On June 2 the analyses of four cultures in each series gave the following results:

						Average
Aerated series	0·18	0·08	0·22	0·22	% CO_2	0·18
Sealed ,,	10·7	10·5	15·6	8·8	,,	11·4
Control ,,	0·64	0·65	0·44	0·50	,,	0·56

It is evident that there is some leakage taking place, since much higher CO_2 concentrations were recorded in the experiment with the sealed pot, the results of which are given above (Table III). It appears probable that in the sealed series under discussion the leakage actually took place through the plant, since repeated observations on sealed pots without plants have shown that the CO_2 concentration rises to 15–20 per cent. in four days and to over 25 per cent. in eighteen to twenty-one days.

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[1] The writer wishes to express his indebtedness to Mr. B. D. Bolas for assistance with this experiment and also Experiments 4, 5, and 8 of the water-culture series.

However, in spite of the leakage, the concentration of CO_2 approaches that which was shown, in the supplementary experiment quoted above, to cause maize to wilt in a few days. The results of that experiment were confirmed in the present instance, when the whole of the plants in the sealed series became unhealthy and soon died. The other two series were continued until July 19, when the plants of each set were harvested, dried, and weighed altogether. The dry weights are shown in Table IV.

TABLE IV.

Average Dry Weight in grm. per Plant of Maize Cultures under different Conditions of Soil Aeration.

Series.	*Shoots.*	*Roots.*	*Whole Plant.*
Aerated	3·88	0·87	4·75
Control	3·50	3·90	4·40

In this case the aerated series again showed an increase in dry weight over the control series, but the increase was much less than in the previous experiment. The CO_2 content of the soil gas was higher in the control series than in the earlier experiment, and probably the same remark applies to the aerated series. Doubtless the nature of the soil in the two cases was contributory to, if not wholly responsible for, the observed difference in CO_2 concentrations. The two series of maize cultures which have been dealt with during 1920 and 1921 indicate that this plant in soil-culture is sensitive to aeration of the soil, but that a CO_2 concentration of 1·8 per cent. in the soil air is no more harmful to it than 0·3 per cent.

II. *Water-cultures.* Concurrently with the soil cultures recorded above, water-culture experiments have been carried out with the same end in view. It is recognized that the behaviour of plants in water-culture is not necessarily an indication of their behaviour when growing in soil, but it was intended that the water-culture experiments, which involve less labour than soil cultures, should be supplementary to the latter. Several series of experiments have been conducted, the conditions of which have differed only slightly, and cultures have been run concurrently with the soil-cultures already described.

Experiment 1. Maize (Barr's Golden Bantam) was used here, the seed coming from the same sample as that used in the pot-cultures of 1920. Narrow-necked milk-bottles of 1,200 c.c. capacity were used as culture vessels. These were arranged in series in parallel rows and the positions were frequently changed in regular rotation. The seeds were soaked overnight in water and sown in sphagnum on March 22 after having been germinated on wet sand. On March 29 the seedlings were set up, one to each vessel, in Shive's three-salt culture solution (17), but this was apparently not suited to the plants, which were soon transferred to a solution as used by Brenchley at Rothamsted, the constitution of which was:

KNO_3	1·0 grm.
$MgSO_4$	0·5 ,,
$CaSO_4$	0·5 ,,
NaCl	0·5 ,,
KH_2PO_4	0·5 ,,
$FeCl_3$	trace
Water	1,000 c.c.

One series of sixteen plants was aerated by bubbling air slowly but continuously through the culture solution, and a second series was grown without the aeration treatment. Several attempts were made to render the aeration more efficient than that obtained by passing large bubbles of air through the solution. The size of the air-delivery tube was varied from the usual 3–4 mm. aperture of the tubing to a fine jet. Also pieces of boiled cane were tried in order to produce minute bubbles. This last method doubtless results in the most efficient aeration, but the control of the rate of bubbling from cane when twenty or thirty bottles are running in parallel is difficult, and eventually no attempt was made to reduce the size of bubbles, the air being delivered from the normal aperture of the tube. Under these conditions regulation is a simple matter. On April 13 the length of the longest root of each plant was measured, with the following results:

Average root length of aerated plants: 35·3 cm. ± 0·8.
,, ,, ,, ,, unaerated ,, 34·4 ,, ± 1·0.

At this stage therefore there was no significant difference in the root lengths of the two series. Possibly owing to the change of culture solution, these two series did not make satisfactory growth, and on May 28 they were harvested, dried, and the plants of each series were weighed *en masse* instead of individually. The weights are given in Table V.

TABLE V.

Dry Weight (in grm.) per Plant of Maize in Water-culture.

Series.	*Shoots.*	*Roots.*	*Whole Plants.*
Aerated	0·98	0·31	1·29
Control	1·02	0·34	1·36

The differences between the two series are undoubtedly within the limits of the experimental error, although no calculation of the error is possible. This experiment indicated that maize, although apparently sensitive to soil aeration in pot-culture, did not respond to aeration of the culture solution when grown in water-culture. Further experiments with maize were deferred until 1921 in order that they could be conducted concurrently with further soil-cultures, and other water-culture experiments were carried out using wallflowers.

Experiment 2. It was thought that the roots of plants in water-culture might be efficiently aerated by growing in the solution a small sprig of a water plant, such as *Elodea canadensis.* In daylight the oxygen produced during photosynthesis by this plant escapes in very small bubbles, and it was considered that a slow succession of these bubbles would oxygenate the culture solution, and at the same time the CO_2 in solution would be removed. As a preliminary, a series of twelve cultures was set up, each consisting of six plants, in wide-mouthed jars. Six of the jars were of earthenware and six of glass, and thus the roots of thirty-six plants were in darkness and the remainder in the light. The plants were held by cotton-wool plugs in holes in a disc of thick cardboard covering the mouth of the jar. Into each of the six glass jars a small sprig of *Elodea* was introduced. Three cultures (eighteen plants) of each series were also aerated twice daily by blowing air through the solution for a few minutes by means of a rubber bulb. This method was not regarded as being entirely satisfactory, but at the time it was the best available. The seedlings were selected for uniformity from a large batch, and were set up on August 23, 1920. The solution was of the same composition as in the previous experiment and was renewed frequently, and also the positions of the cultures were changed in rotation. Leaf counts were made at intervals, but after a time it became evident that the number of leaves was not a real guide to the size of the plant, since the leaves of the plants aerated by *Elodea* were noticeably larger than those of the other cultures. The results of the leaf counts are given in Table VI.

TABLE VI.

Total Number of Leaves in Series of Eighteen Plants of Wallflower, in Water-culture. Solution Aerated in Various Ways.

Date.	*No Elodea, Roots in dark.*		*Aerated by Elodea, Roots not darkened.*	
	Control.	Additional Aeration.	No additional Aeration.	Additional Aeration.
August 29	125	131	127	129
Sept. 1	141	143	138	145
Sept. 5	161	165	164	171
Sept. 9	172	182	181	189
Sept. 13	190	202	200	211

The probable error of these figures is approximately 2 per cent. The leaf number shows a tendency to increase as the amount of aeration increases, being 10 per cent. greater in the fourth series than in the control, and with this must be coupled the observation that the leaves were larger in the fourth series than in the control. This observation was confirmed by the dry weight results when the plants were dried and weighed on September 27. Each culture of six plants was weighed separately, and thus the average weight for each series represents only three weights of six plants each (Table VII). This doubtless accounts for the large probable error.

TABLE VII.

Series.	*Dry Weight.* grm.
Control	0·89 ± 0·12
Roots in dark, aerated by bulb	1·04 ± 0·17
Roots in light, aerated by *Elodea*	1·36 ± 0·12
,, ,, ,, aerated by both methods	1·27 ± 0·06

Owing to the small number of cultures it is perhaps inadvisable to generalize from these results, except to point out that the differences between the first series and the third and fourth are significant. The results were sufficiently striking to warrant a further experiment on the same lines, which was therefore initiated in spite of the lateness of the season.

Experiment 3. The culture vessels in this experiment were the same as in Experiment 1, 1,200 c.c. milk-bottles, and the solution used was also the same. Each vessel contained one plant. The cultures were set up on October 1 in three series of thirteen each. Series A was a control, the culture vessels being covered with black paper. The vessels in Series B were also covered, and air was bubbled continuously through the solutions as in Experiment 1. Series C had no covers on the bottles, so that the roots were in the light, and a little *Elodea* was introduced into each. The cultures were grown for fifty-six days, but the growth was small owing to the season. At the time of harvesting, root length was measured and the leaves were counted. Each plant was weighed separately. The results are shown in Table VIII.

TABLE VIII.

Wallflower in Water-culture, under different Conditions of Root Aeration.

Series.	*Root Length.* cm.	*No. of Leaves.*	*Dry Weight.* mg.
A. Control	19 ± 0·9	24 ± 0·3	105 ± 3·6
B. Aerated continuously	15 ± 0·6	24 ± 0·4	116 ± 3·4
C. Aerated by *Elodea*	15 ± 0·4	24 ± 0·6	137 ± 4·7

The difference in length of root is marked, and it has been observed in every case in which plants have been grown in water-culture with their roots in the light that these roots are much shorter than the roots of cultures in darkened bottles. The roots which have been in the light also have a greater dry weight than roots in the dark, as will be seen in later experiments, but it is certain that some of this increase is due to the accumulation of green algae, which is usually present and which cannot be entirely removed. In the present experiment the quantity of algae, for some unknown reason, was very small. The account given by Cannon and Free (6) of the changes of root form which accompany changes of aeration condition, suggests that short thick roots are produced under anaerobic conditions. In the present experiments they were produced under conditions of more, rather than less aeration. The light may have been the active factor in preventing growth in length in Series C and in experiments described below, but not in Series B.

The dry weights of the plants again show an increase as the result of aeration by *Elodea,* whilst the increase due to continuous aeration is of doubtful significance. The leaf numbers were identical in all three cases, and since the weight is greater in the aerated series, it is again emphasized that the size of the leaves is greater. This confirms the observation in the previous experiment.

Experiments 4 and 5. In 1921 two series of water-culture experiments were conducted concurrently with the pot-culture series, maize (Giant Caragua) from the same sample being used. These two experiments were very similar, and may be dealt with together. In Experiment 4 the plants were growing in the 1,200 c.c. narrow-necked vessels, and the growth period extended from March 1 till April 25. In Experiment 5 the culture-vessels were wide-mouthed jars of 1,000 c.c. capacity, and the growth period covered 54 days from March 6 to April 29. The culture solutions and general treatment were the same as in the previous experiment. No measurements of root length were made, and no leaf counts were taken. At the close of the experiment the plants were gathered, and dried and weighed separately. The average dry weights are shown in Table IX.

TABLE IX.

Dry Weight (in grm.) of Maize in Water-culture under different Conditions of Root Aeration.

Expt.	*Series (13 plants to each).*	*Shoots.*	*Roots.*	*Whole Plant.*	$\frac{\text{Root}}{\text{Shoot}}$ *Ratio.*
4.	Control	0·78 ± 0·06	0·31 ± 0·02	1·09 ± 0·08	0·40
	Aerated continuously	0·69 ± 0·05	0·28 ± 0·02	0·97 ± 0·07	0·41
	Aerated by *Elodea*	0·71 ± 0·05	0·49 ± 0·03	1·21 ± 0·07	0·69
5.	Control	0·75 ± 0·05	0·30 ± 0·02	1·05 ± 0·07	0·40
	Aerated continuously	0·79 ± 0·05	0·30 ± 0·02	1·08 ± 0·07	0·38
	Aerated by *Elodea*	0·86 ± 0·05	0·46 ± 0·09	1·32 ± 0·09	0·54

In this table the weights of the roots are included, but it should be emphasized that the weight of roots grown in the light include a considerable quantity of algae, and it is uncertain how far these are responsible for the higher weight. Consequently, in the case of the series aerated by means of *Elodea*, only the shoot weights can be considered reliable. The difference in root weight is clearly brought out in the last column, where the ratio of root to shoot is shown to be practically identical in all series where the roots were darkened. In the series with roots in the light the ratio is considerably increased. It was again observed in these two series that the roots of aerated plants were much shorter than in the control series.

Apart from the points just considered, there is no difference between any of the series which can be said to be significant, and these two experiments confirm the indication given by the maize experiment in 1920, that this plant does not respond to aeration in water-culture, although it has been demonstrated that when the plant is grown in soil, an increase in dry weight results from aeration.

Experiment 6. A further experiment with wallflowers was carried out in 1921. The cultural conditions were quite similar to those in previous experiments, the plants being grown in 1,000 c.c. vessels for 36 days. Only two series were set up, one of which was aerated by *Elodea*, and the other acted as a control. Sixteen plants constituted the control series and thirteen the aerated series. No distinction was made between roots and shoots. The average dry weights per plant were as follow:

Control series	0·64 ± ·02 grm.
Aerated series	0·88 ± ·05 „

The difference of 38 per cent. is doubtless partly accounted for by the algae on the roots of the aerated series.

Experiment 7. In this case seedlings of *Chenopodium album* were used, and the cultural conditions were similar to those of previous experiments. The duration of the experiment was 29 days (June 7–July 6). Two series of six cultures, each culture containing two plants, were set up, one series being aerated by *Elodea* and one serving as a control. The plants grew well and uniformly, so that in spite of the small number of plants the probable error of the averages proved to be quite low. Table X gives the average dry weights.

TABLE X.

Dry Weight (in grm.) of Chenopodium in Water-culture under different Conditions of Root Aeration.

Series.	*Shoots.*	*Roots.*	*Whole Plants.*	$\frac{\textit{Root}}{\textit{Shoot}}$ *Ratio.*
Control	0·92 ± 0·06	0·19 ± 0·01	1·11 ± 0·07	0·21
Aerated by *Elodea*	1·36 ± 0·06	0·42 ± 0·01	1·78 ± 0·07	0·31

In this case again the ratio of root to shoot has been increased 50 per cent. in the aerated series, and at the same time the shoot weight was increased nearly 50 per cent. This plant responds to a marked extent to aeration in water-culture.

The experiments which have been recorded in this paper show that maize may respond to aeration of the soil in which it grows, but it has not been found possible to demonstrate any increase in growth of maize in water-culture as the result of aeration. *Cheiranthus* and *Chenopodium* in water-culture gave greater yield of dry weight as a result of aeration of the culture solution. No analysis of the cause of this increased yield has been attempted. It may be due to oxygenation of the solution or to the removal of respired CO_2, but on the other hand it is possible that another factor may be concerned. Aeration of a water-culture solution by bubbling a stream of air through it, results in a very thorough circulation within the solution. The consequence of this is that not only is any solid matter present, such as phosphates, kept in movement and distributed throughout the solution, but also local concentrations or dilutions of nutrients, due to the absorption by the growing roots, are prevented. Thus the roots are supplied with a nutrient medium representative of the whole solution instead of being dependent upon diffusion for the renewal of salts removed locally by absorption. In the absence of any data with regard to local concentrations in nutrient solutions, this incidental stirring effect of aeration can hardly be neglected.

The response of a plant to soil aeration must be determined by two factors (apart from any toxic effect of CO_2), the quantity of oxygen required by the roots and the quantity of oxygen available. This latter factor is dependent on the rate of diffusion through the soil and also on the rate at which oxygen may pass to roots through the internal aeration system of the plant. Thus, a plant with a large oxygen requirement may grow satisfactorily in a soil with a low oxygen supply, owing to its capacity for internal conduction of air. Such a plant could not be expected to respond by more rapid growth to additional soil aeration, and the failure of the plant to respond to soil aeration may thus be due to the fact that it is not sensitive to the treatment. This is hardly likely to have been the case with maize, which showed a response to soil aeration when grown in soil-culture. Lack of response may also be due to insufficiency of aeration. It is possible that even the continuous stream of bubbles which was maintained in water-culture experiments, was insufficient to reduce the CO_2 content, or to increase the oxygen content of the nutrient solution to a point at which an effect on growth was produced. A further possibility is that, although the plants may be sensitive to root aeration, the conditions of water-culture were such that the plants were unable to respond, owing to the limiting effect of some other factor. For example, it may be impossible, owing to the slight solubility of oxygen, to raise the oxygen content of the culture solution sufficiently to influence appreciably the rate of oxygen absorption by the roots.

The influence of daily aeration of the culture solution by *Elodea* is noteworthy. Whilst these experiments were in progress it was found that Bergman (3) had adopted a similar method of aeration, and found that day aeration by means of *Elodea* was effective in increasing oxygen content and decreasing CO_2 content of the culture liquid. In the present experiments it was intended that the aeration should be brought about by *Elodea*, but in effect the algae which grew on the walls of the vessel and on the roots of the plants, owing to the culture vessel not being darkened, doubtless affected the oxygen and CO_2 content of the solution. The differences in root dry weight which have resulted from aeration by *Elodea* have been rejected in considering the above records of experiments owing to the presence of an unknown weight of algae, but examination of these roots was sufficient to convince the writer that the quantity of algae was quite inadequate to account for the large increase of root dry weight which was sometimes observed, and that therefore a real increase had occurred. Whether this increase and the increase in shoot dry weight were due to aeration or whether the exposure of the roots to light had any effect has not been determined. An additional complication arises from the fact that *Elodea* and algae are absorbing small quantities of nutrient material from the culture solution, and although this factor probably acts deleteriously towards the culture plants, it must be recognized that the difference in the cultural conditions in series aerated by water-plants, and control series is not only a difference of aeration.

SUMMARY.

Maize in soil-culture shows an increase in dry weight if the soil is aerated. The CO_2 content of the atmosphere of a loam increases rapidly if the soil is in a sealed pot, reaching 1 per cent. in two hours and 34 per cent. in twenty-three days.

In water-culture maize failed to respond to aeration of the nutrient solution.

Wallflowers and *Chenopodium album* showed considerable increase in dry weight as the result of aeration. A similar increase followed aeration of the culture solution during daylight by means of *Elodea*.

The increase in weight in wallflowers was due to an increase in leaf size.

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The Spermatia of Rhodymenia palmata, Ag.

BY

E. MARION DELF, D.Sc., F.L.S.,

Late Yarrow Fellow of Girton College,

AND

VIOLET M. GRUBB, M.Sc.

With four Figures in the Text.

OWING to the minute size and very limited seasonal periodicity of the male reproductive organs of the Florideae, the spermatia of this group are less well known than either the procarps or the tetraspores, and, indeed, complete descriptions of them are only to be found for comparatively few species.

In the case under consideration, the well-known red alga *Rhodymenia palmata*, though the spermatial fronds have previously been often sought for among the masses of material found on exposed rocky shores in the Northern Hemisphere, there are only two isolated records of their discovery and recognition. Thuret (12), in an article published in 1855, gives a figure of the transverse section of a male thallus, accompanied by a short note in the text to the effect that he has found male fronds in the winter, and on them the antheridia are arranged in pale sori[1] of irregular shape. This figure is reproduced by Oltmanns (8, 9), but with no comment on it or mention of the spermatia in the text. Buffham (1), forty years later, records the fact that he found several plants at Swanage in June, bearing dingy green patches, and these in section show elongated cells, while he adds: 'I cannot but think it probable that these curious patches of so-called antheridia have no fecundating corpuscules, and they may, indeed, be a case of degeneration from progenitors possessing the necessary capacities.'

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[1] Strictly speaking, this term should be reserved for asexual reproductive organs such as the sporangia of ferns. In practice, however, it is convenient and, we think, not misleading to use the term for more or less definitely organized groups of sexual reproductive organs such as those found in the red algae. A similar licence is taken by Svedelius in describing the spermatia of *Delesseria sanguinea* (Svensk Bot. Tid., 1912, Bd. vi, 239).

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While visiting Shanklin, Isle of Wight, in February 1923, the authors first came across the male fronds when examining some fresh material of *Rhodymenia palmata.* Having once recognized the fronds, it was found on returning to the shore that they were present in large numbers and were quite as prevalent at that season as the tetrasporic plants; out of about 300 plants taken at random from different parts of the shore and examined, quite one-third were found to be bearing spermatia. On revisiting Shanklin in April the fronds were still present, though noticeably fewer in number. Fronds have been found in other places, such as Broadstairs, where the young male thalli were only just developing in March; this may be accounted for by the greater exposure of the shore as compared with the ridge of rock at Shanklin. Also at Swanage there was no difficulty in finding the fronds in the month of April, though these had disappeared in June, while material sent from Guernsey in March and from the north coast of Cornwall in April showed the presence of male fronds there. It appears therefore that the spermatial fronds are developed for a short season

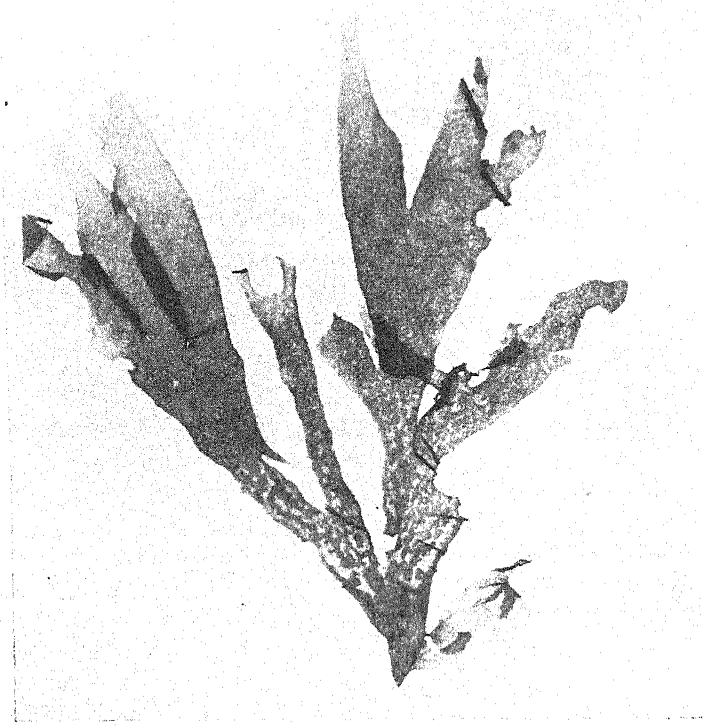

FIG. 1. Photograph of a spermatial frond of *Rhodymenia palmata* showing the irregular pale sori on the lower portions of the thallus, the apices being non-fertile. (This photograph was kindly taken for us from one of our herbarium specimens by Mr. J. Ritson.)

beginning in February or even earlier and closing in May or June according to locality.

The spermatia occur upon male plants, and in outward form, size, &c., the latter do not differ in any way from the sterile ones. The sori in which the spermatia are developed are on either or both surfaces of the thallus, and are present as small pale patches or as large irregular developments covering the surface of the frond with the exception of the apex, which, in our experience, is never fertile (Fig. 1).

The sori, which are very similar to the easily recognized tetrasporic patches found on other plants, can soon be distinguished by their paler colour, their lack of any hard definite outline, and by the fact that they are not raised above the surface of the frond to the same extent as the tetrasporic sori. Often every frond on a spermatial plant may be found to be covered with sori; at other times some of the fronds are sterile, while in at least one case a plant has been found bearing spermatia on some fronds and tetraspores on others.

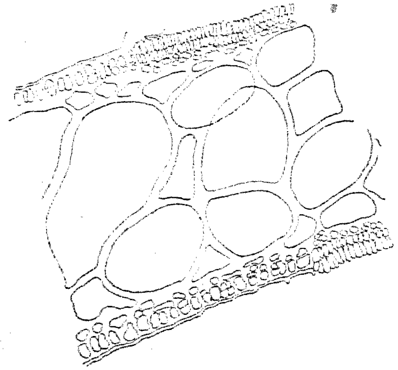

FIG. 2. Outline drawing of a transverse section of a frond showing the formation of young spermatial sori by means of the divisions of the superficial cells of the thallus. × 230.

Fronds were fixed on the shore in various ways in order to avoid shrinkage of the tissues. When necessary to preserve material for examination and sectioning, a mixture of 50 per cent. alcohol and 50 per cent. glycerine in equal proportions was found to be the most effective. For microscopic examination, apart from cytological observations, it was found that the best results were obtained from hand-sections stained and mounted in pure glycerine, rather than from microtomed sections in Canada balsam. No matter how carefully the process of dehydration in microtoming was carried on, the gelatinous walls of the antheridia invariably shrank and sections similar to those represented in Fig. 4 resulted (cf. Fig. 3, hand-sections mounted in glycerine). This shrinkage of the walls no doubt accounts for the difficulty many workers on spermatia have had in deciding where walls are actually present, and the shrunken condition can indeed often be seen in figures of spermatia (11, p. 239).

A transverse section through a vegetative frond shows that the thallus consists of a central axis of several rows of large cells, with one or more layers of superficial cells containing plastids, the whole being covered by a firm gelatinous membrane.

In a young male frond where there is only a single row of superficial cells, the first sign of the formation of a sorus consists in the transverse division of these cells over a large or small area (Fig. 2); of the two cells thus formed, the lower one, which is more deeply pigmented, forms the basal cell of the antheridial group, and the upper one the antheridial mother-cell,[1] which gives rise to the antheridia containing the spermatia (Fig. 3, *a*). In an older frond where transverse divisions have already taken place in the superficial cells, the two uppermost rows of cells over a soral area become

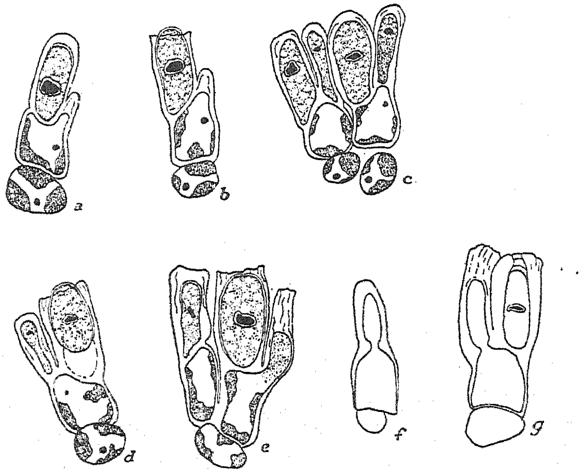

FIG. 3. Camera lucida drawings, showing the formation of spermatia. × 1200.
a. Basal cell and antheridial mother-cell. From the latter, one fully-formed antheridium has been developed, and another (right) is forming.
b. Ripe spermatium about to escape from ruptured gelatinous sheath (i.e. antheridium).
c. Two antheridial mother-cells, each giving rise to two antheridia.
d. Escaping spermatia (right), showing the cavity left at the base of the gelatinous sheath before the latter has shrunk back. Left—a young antheridium.
e. Stages in development of secondary antheridia.
f. Primary spermatium being constricted off from the protoplast of the antheridial mother-cell.
g. Left—a secondary antheridium arising within the old antheridial wall. Right—a section passing through a developing antheridium, showing the thick gelatinous walls left by previous antheridia.

the basal cells and the antheridial mother-cells respectively. Each antheridial mother-cell is somewhat two-sided at the apex and possesses a thick gelatinous wall. On the formation of a spermatium a small bud is put out at one side of the mother-cell; this bud increases in size, stretching the gelatinous wall until it is about equal in length to the cell from which it arose (Fig. 3, *a*). An oblique wall is then formed as a ring-like ingrowth, constricting off the protoplast (Fig. 3, *f*), which appears as an oblong colourless body, apparently naked and vacuolate; this is the young spermatium within the wall of the antheridium. As the spermatium ripens it becomes larger, growing more nearly spherical, though often rounded at the apex and pointed at the base (Fig. 3, *c*). Finally the gelatinous wall ruptures, apparently by internal pressure (Fig. 3. *b*, *d*), and the spermatium escapes, leaving the outer wall behind as a shrunken sheath with a torn and ragged aperture (Fig. 3, *e*). Hand-sections from fresh or from unshrunken preserved material show clearly that the spermatium is distinctly clothed in its own gelatinous wall[1] (Fig. 3, *b*, *d*).

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[1] The terminology applied to the groups of cells which in the red algae ultimately give rise to spermatia is somewhat vague. We have in the main adopted the nomenclature in use by Oltmanns (8, 9), as it appears to be more in accordance with the usage in other algal groups. The term antheridium is therefore given to that cell in which the spermatium is formed and from which it escapes. The cell subtending the antheridium (or antheridia, as the case may be) is termed the antheridial mother-cell (the 'Spermatangiummutterzelle' of Svedelius, 10), while the sister-cell beneath it is referred to as the basal cell.

More than one antheridium is, however, developed from the antheridial mother-cell, for while the first is maturing a second is gradually forming by its side (Fig. 3, *b*). At the time of the discharge of the first, the second one is just going to be, or has just been, constricted off from the opposite side of the antheridial mother-cell, and it in turn produces a solitary spermatium which escapes through an irregular opening in the apex of the antheridium.

In the meantime a new antheridium has developed within the old ruptured sheath of the first one, and a new spermatium, breaking through its antheridial wall, slips out through this sheath (Fig. 3, *e*, *g*). How often this alternate process in the formation of antheridia is repeated is not known, as it is impossible always to tell how many spermatia have slipped out through the original sheaths on either side; observation shows that each antheridial mother-cell can produce in alternate succession at least four spermatia—two on either side of the apex.[2] No amoeboid movement was seen in freshly discharged male reproductive cells when examined in sea-water.

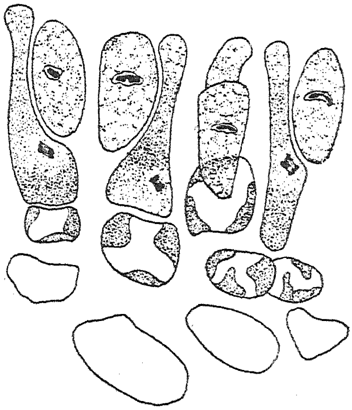

FIG. 4. Camera lucida drawing of a transverse section through a spermatial sorus. The nuclei of the antheridial mother-cells are seen undergoing karyokinetic division. In the ripe spermatia the nuclei are seen in the characteristic elongated condition. Gelatinous walls of the antheridia not visible. Microtomed section. × 1800.

The spermatium nucleus is formed as the result of the division of the single nucleus of the antheridial mother-cell, which takes place shortly before the spermatium is constricted off (Fig. 4). One of the daughter nuclei passes into the spermatium and lies at first near the apex, the cytoplasm at this point being distinctly vacuolate. Later the nucleus may take up a central position, becoming there surrounded by vacuolate cytoplasm. When the spermatium is discharged the nucleus is usually curved and curiously elongated (Fig. 4), sometimes appearing as though in the late prophase of a karyokinetic division. The subsequent behaviour of the spermatium has not been investigated.

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[1] See discussion, p. 333.

[2] Compare p. 332.

In a former communication by one of us, it was suggested that the mucilaginous hairs found in small patches on the fronds of *Rhodymenia palmata* in the early months of the year were of the nature of trichogynes, although mature carpospores had not then been found (3). It was also suggested that certain minute bodies found attached to the hairs might prove to be spermatial in nature; it seems, however, doubtful whether these bodies can be identical with the undoubted spermatia now described. No further evidence has been found of mature carpospores, and the suggestion is therefore made that a former sexual phase has been partially lost in this alga.

DISCUSSION.

The development of the spermatia of *Rhodymenia palmata* follows in the main that of the higher Florideae already described by other observers (7, 10, 11). Certain details in the development are, however, worthy of further comment:

(a) *Successive Formation of Antheridia.*

A study of the available literature clearly shows that in the higher Florideae the antheridial mother-cell usually forms more than one antheridium. Their number and position, however, vary in different genera.

Thus in *Martensia*, Svedelius (10) states that a single antheridium is constricted off at the apex of a mother-cell and a new one is formed in the same position when the first has been shed. In *Melobesia*, terminal antheridia are constricted off successively, like conidia, before the release of those first formed (4, Pl. VI, Fig. 22, and 9, Fig. 571).

On the other hand, in the Ceramiales, a slightly lateral position is more frequent, the antheridia being constricted off obliquely right and left of the apex; this has been described and figured by Svedelius for *Delesseria sanguinea* (11). Yamanouchi (14) figures a very similar development for the 'sperms'[1] of *Polysiphonia violacea*, and Kylin (7) states that in *Laurencia pinnatifida* the mother-cell gives rise to 'three or four spermatangia'.

According to Oltmanns (9) spermatangia (i.e. antheridia) in *Griffithsia* are sometimes seen growing up through the empty wall of an older cell (7 and 9, Fig. 571), so that at least three or four antheridia would then be formed from one mother-cell.

In *Rhodymenia palmata*, Fig. 3, *g*, shows a thick lamellate wall open at the top, surrounding the newly-formed antheridium. This wall appears to be the empty gelatinous sheaths of the two previously formed antheridia, from which the spermatia have been discharged. If this interpretation is correct, it seems probable that the formation of new antheridia from one mother-cell may continue indefinitely.

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[1] The so-called 'sperms' of Yamanouchi are equivalent to the antheridia of the present account, and to the spermatangia of Kylin and Oltmanns, since in *Polysiphonia* the whole antheridium is said to become detached, and with its contents to function as a spermatium.

(b) *The Wall of the Spermatium.*

The spermatia of the Florideae have been variously described as naked protoplasts (Schmitz, Svedelius, Kylin) or as clad in a delicate cell-wall (Guignard, Yamanouchi). The following are said to have naked spermatia:

Nemalion multifidum—Cleland (2), 1919.
Batrachospermum moniliforme—Kylin (6), 1917.
Delesseria sanguinea—Svedelius (11), 1912.
Rhodomela virgata—Kylin (5), 1914.
Polysiphonia nigrescens—Kylin (7), 1922.

The following species are reported as having spermatia clothed with a delicate cell-wall:

Polysiphonia violacea—Yamanouchi (14), 1906.
Melobesia membranacea—Guignard (4), 1889.
Corallina officinalis—Yamanouchi (15), 1921.
Rhodymenia palmata—Delf and Grubb (this paper).

Kylin (5 and 7) believes that the spermatium most often slips out from the antheridium as a naked protoplast. After escape, it 'sooner or later' appears to have acquired a very thin wall. Kylin, however, states that it is possible that in some cases this thin membrane may be the innermost layer of the wall of the antheridium, which may be carried away in the escape of the spermatium.

Yamanouchi, on the other hand, challenges this view (15), stating that a careful re-investigation would reveal the presence of a thin cell-wall around the spermatium in *Delesseria sanguinea*, and also probably in other cases. It certainly seems unlikely that the two species of *Polysiphonia* quoted above should differ in this respect.

In our experience the wall around the spermatium of *Rhodymenia palmata* is only clearly seen in material which has been carefully handled to avoid shrinkage; but it is impossible to say whether the wall is the innermost layer of the wall of the antheridium or a new wall formed around the spermatium.

(c) *The Nucleus of the Spermatium.*

The spermatia of the Florideae appear to be invariably uninucleate (cf. 9, p. 372).

In some cases, the nucleus appears to be in the resting condition, which in *Lemanea*, according to Kylin, is prolonged until after the escape of the spermatium (7); but more often the nucleus appears to contain a number of chromatin granules ('Körnchen'), as though in the prophase of division. In *Polysiphonia violacea* these granules are twenty in number, and Yamanouchi (14) considers that they represent the chromosomes of the typical gametophytic nucleus. The nucleus of the spermatium of *Delesseria sanguinea* also has twenty chromatin granules, and Svedelius (11) describes this as a late prophase of nuclear division in which the haploid number of chromosomes appears.

In *Rhodymenia palmata* the curved nucleus appears to have two aggregates of chromatin separated by a narrower band. The whole is surrounded by a paler area, and suggests somewhat a curved nucleus in prophase with aggregated 'Körnchen' (Fig. 4).

These indications of nuclear division are of some interest, since it was shown by Wolffe in 1904 (13), and confirmed by Cleland in 1919 (2), that in *Nemalion* the nucleus of the spermatium divides into two when the spermatium is attached to the trichogyne, only one of the daughter nuclei taking part in fertilization. This division appears to be tentatively initiated but never completed in the spermatia of the majority of the higher Florideae hitherto investigated.

(d) *The Homology of the Spermatium.*

In most cases the Floridean spermatium is described as formed from the whole contents of the antheridium. Only Svedelius in *Delesseria sanguinea* (11) describes and figures spermatia in the formation of which part of the cytoplasm of the antheridium remains unused. In a more recent account of the same species, however, Kylin (7) states that the *whole* contents of the antheridium escape.

Appearances similar to those figured by Svedelius have been observed by us in *Rhodymenia palmata*, but seem to be merely optical illusions. In every case where a spermatium is seen to have escaped, the wall of the antheridium remains behind as an empty shell (Fig. 3, *e*).

Assuming that the whole contents of the antheridium go to form the spermatium, the latter has been compared with the single spermatozoid which slips out of the antheridium in *Coleochaete* as a naked motile gamete, but this comparison obviously breaks down if the observations recorded in section (b) are correct. The spermatium in the Florideae must then be of the nature of an antheridium, as has indeed already been suggested by Yamanouchi (14). This theory lends support to the view that the membrane round the spermatium is itself the inner layer of the antheridial wall (cf. p. 333).

SUMMARY.

1. Spermatial fronds of *Rhodymenia palmata* have been discovered and

2. These fronds are developed from February to June in different localities, and closely resemble those of sterile or tetrasporic plants.

3. The male fronds are covered on both sides with flat irregular patches of sori.

4. The young sorus consists of numerous antheridial mother-cells, superficial in origin and each subtended by a basal cell.

5. Each antheridial mother-cell buds off alternately right and left from its apex an antheridium containing a solitary spermatium.

6. Each spermatium, when ripe, is extruded from the torn apex of the antheridium, and is seen to be surrounded by a delicate membrane.

7. In *Rhodymenia palmata* it has been shown that the male reproductive organs are fully developed, the female being perhaps vestigial.

WESTFIELD COLLEGE, LONDON.
December 1923.

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The Ovule and the Development of the Female Gametophyte of Macrozamia Fraseri.

BY

K. E. LIGHT, B.Sc.,

Assistant Lecturer and Demonstrator at the Royal Holloway College, University of London.

With Plate X and twenty-six Figures in the Text.

INTRODUCTION.

OVULES of *Macrozamia Fraseri* were examined for the general structure of a cycadean ovule. The material had been collected by Professor M. Benson in the latter part of the summer of 1914 at Fremantle, Australia, while on the British Association Expedition. A few ovules only were available, but they were in a young condition, showing several stages in the development of the female gametophyte. The stages obtained were, however, those ranging only from free nuclear division to a sac filled with cellular endosperm. In the oldest condition no archegonia had developed. A further examination showed that those ovules in which the endosperm was in the free nuclear stage were very well preserved. All the typical stages in mitosis were found, even in one sac, and the transitional figures presented some interesting points.

The material had been fixed in alcohol as soon as possible after the cone had been collected. The free nuclear stage alone showed good preservation as regards any cytological details.

Reference to the literature on the Cycads showed that not only was *Macrozamia* one of the least investigated of the Cycad genera, but the records of the early development of the endosperm for any genus were very fragmentary. The following account will deal with these points in particular:

1. The development of the endosperm.
2. Certain details of mitosis.

As regards the female gametophyte of *Macrozamia*, Chamberlain (1) has described female cones of *Macrozamia Moorei*, but the endosperm had

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reached its full size and showed archegonia. In other genera, a row of megaspores has been figured for *Ceratozamia* (2), *Zamia* (3), and *Stangeria* (4), the functional megaspore being the first cell of the female gametophyte. In *Stangeria* Lang showed the subsequent growth of the megaspore, the gametophyte being represented by a layer of cytoplasm with numerous nuclei: no stage between that and a cellular endosperm being obtained. In *Dioon* Chamberlain (5) found ovules early in November with a cellular gametophyte. He suggested that this condition had been succeeded by the formation of very large cells which had divided repeatedly. The cells showed no visible food contents, the peripheral layer being smaller than the others. Caldwell (6), working on *Microcycas*, found no early stages, but from the structure of the adult tissue concluded that it had arisen in a centripetal manner. The cells showed a radial arrangement, and a median line was distinct, formed by the abutting tissues.

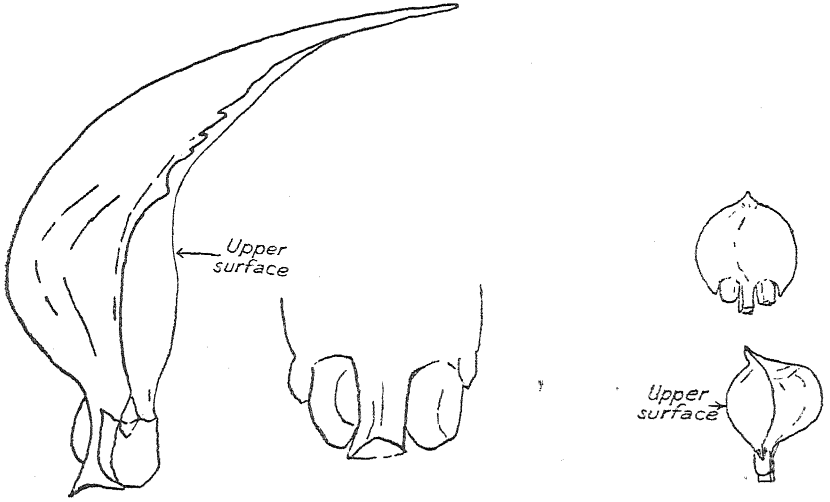
The exact position of our records regarding endosperm formation in the Cycads is stated clearly by Coulter and Chamberlain (7): 'Although cell formation has not been studied among Cycads, it is evident that it begins at the periphery of the embryo sac and advances towards the centre, as has been described for other Gymnosperms.'

THE OVULE OF *MACROZAMIA*.

The only material available for investigation was of *Macrozamia Fraseri* and another smaller species referred to here as *Macrozamia* sp. The ovules, in both cases two in number, are borne on the sporophyll, one on either side of the stalk, as in most genera of the group. Reference to Text-figs. 1 and 2 will indicate the difference between the sporophylls of these two species. In *Macrozamia Fraseri* there is some evidence of the leaf-like nature to be seen in the somewhat serrated margin of the organ, but this is not apparent in the smaller form. Both drawings were made of these species at the time when free nuclear division is occurring in the gametophyte. The sporophylls were hard and woody, forming a close compact cone. The upper and lower sporophylls were sterile.

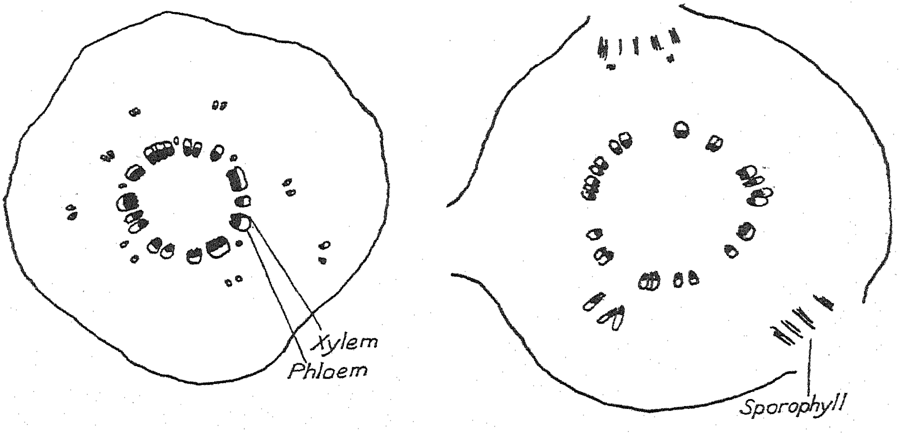
In the smaller species, certain observations were made as regards the anatomy. In the cone peduncle the normal arrangement of the Cycadaceae is present. There is a ring of about ten vascular strands in the median region, each being of the normal endarch collateral type, and only centrifugal wood is present. Traces are given off as single strands in a spiral dividing into two in the cortex (Text-fig. 3). These traces may fuse and do not supply any appendages. The main strands of the peduncle divide and fuse throughout their course, so that the number remains approximately constant.

The peduncle is in direct continuation of the cone axis. In this the structure is similar, but, owing to the vascular supply to the sporophylls, the number of strands in any transverse section is greater. The sporophyll traces show this difference from the traces below in the peduncle. They are double, arising by division of two separate strands of the axis (Text-fig. 4).



1. 2.

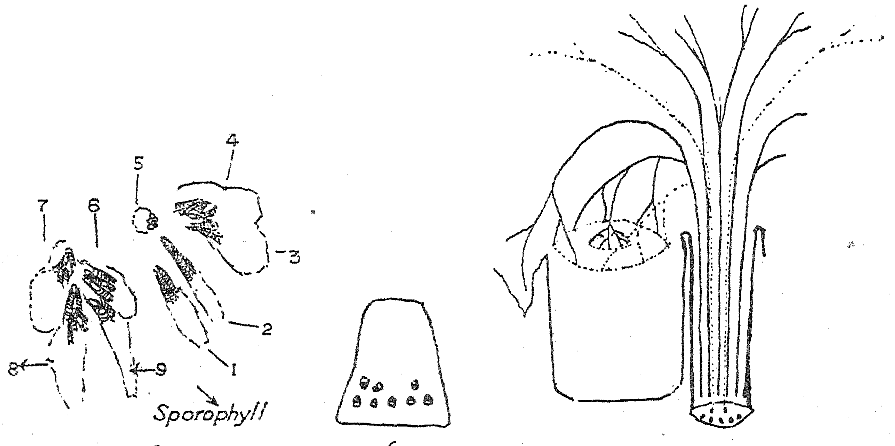
TEXT-FIGS. 1, 2. 1. *Macrozamia Fraseri*, ♀ sporophyll. 2. *Macrozamia* sp., ♀ sporophyll.



3. 4.

TEXT-FIGS. 3, 4. *Macrozamia* sp. 3. Transverse section of peduncle of cone. 4. Transverse section of cone axis.

These traces pass out almost at right angles, since the sporophyll is borne practically at right angles to the axis. The traces divide in the cortex, one bundle dividing soon after leaving the main ring. The division results in the formation of a smaller median strand (Text-fig. 4). Division of the two outer strands takes place, so that the strand, by becoming concentric first, divides, leaving a smaller strand with inverse orientation and two other strands with normal orientation. Thus the sporophyll is supplied with seven strands. Further division was found to occur in some cases; this is figured in Text-figs. 5 and 6, but it may not occur until later in the sporophyll. The outer strands are generally larger than the inner ones. Of the five strands which lie nearer to the abaxial surface, the median one passes up the sporophyll, remaining sterile in that it only supplies the sporophyll. Throughout its course, it gradually becomes concentric, finally dividing into three, the strand nearer the adaxial surface soon ending in the parenchyma. Similarly, the inner strands on either side of the median are also sterile. The two pairs of outer strands, inversely orientated, supply the ovule, as shown in Text-fig. 7, one branch of the adaxial strand being sterile. Thus the ovule is supplied with the double vascular system characteristic of a cycadean ovule (Text-fig. 8).



5. 6. 7.

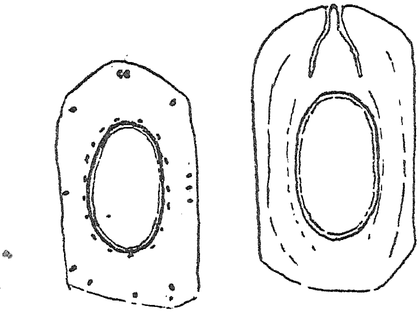
TEXT-FIGS. 5–7. *Macrozamia* sp. 5. Division ot sporophyll traces in cortex of cone axis; traces numbered. 6. Transverse section of stalk of sporophyll. 7. Diagram of vascular supply to ♀ sporophyll.

The vascular supply to the ovule of *Macrozamia Fraseri* was not investigated, as material of the sporophylls was not available. In all probability, though more complex, it will be found to conform to a similar plan.

During the development of the endosperm, the ovule does not appear to undergo much alteration after free nuclear division has advanced considerably. The ovule shows a much elongated nucellus in the micropylar region (Text-fig. 8), but no differentiation has occurred in the integument and no disorganization to form a pollen chamber has taken place in the nucellar apex. The ovules of *Macrozamia Fraseri* measured approximately 1 cm. in length and 0·5 cm. in width, the endosperm forming a sac 0·5 cm. in length and 0·25 cm. in width.

THE DEVELOPMENT OF THE ENDOSPERM IN *MACROZAMIA FRASERI.*

The youngest stage showed free nuclear division of the one functional megaspore in active process. The sac had reached the size given above. There was a large central vacuole and a parietal layer of nuclei embedded in cytoplasm surrounding the sac. It was difficult to make any accurate estimation of the number of free nuclei in any one sac owing to the fact that divisions did not always proceed in a regular way, i.e. division was not always strictly simultaneous. A rough estimation indicated that in all probability in *Macrozamia Fraseri* wall-formation does not occur until after the tenth division—that is, after the formation of 1,024 nuclei.



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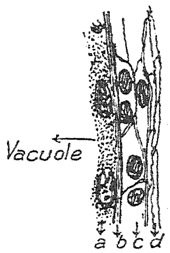


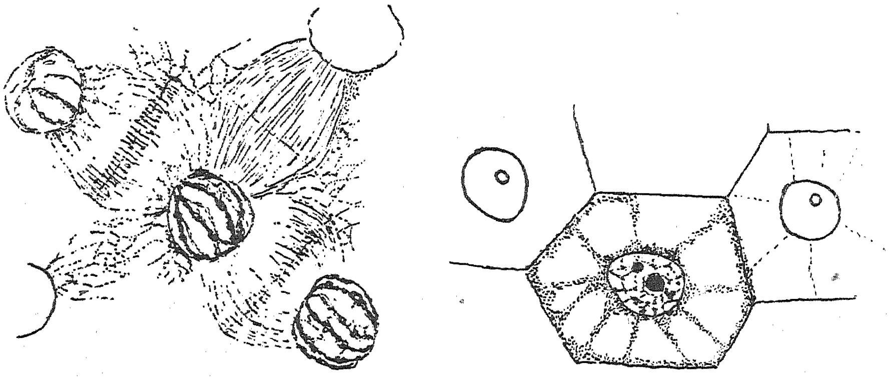
9.

TEXT-FIGS. 8, 9. *Macrozamia Fraseri.* 8. Ovule in transverse (left) and longitudinal (right) section. 9. Development of ♀ gametophyte; free nuclear division. *a*, free nuclei in cytoplasm; *b*, 2-layered megaspore membrane; *c*, tapetal layers; *d*, cells crushed by developing megaspore.

It was this stage, with its hundreds of dividing nuclei, presenting such a striking scene, which led to this investigation. The appearance is shown in Plate X, Fig. 1, where several nuclei are seen in metaphase. This was only a fragment of the whole endosperm. The method employed for this stage was as follows: The sac was removed from its nucellus and integument, opened and laid out on a slide, and stained. The megaspore membrane could be quite easily removed, leaving the thin layer of protoplasm with the dividing nuclei. For later stages in the formation of the endosperm it was found advisable to embed in paraffin and section the material. Various stains were employed, but in general Heidenhain's iron-alum-haematoxylin method and Flemming's triple gave the best results.

In this condition the megaspore membrane is very difficult to section, but after prolonged infiltration of paraffin it was evident that it is a two-layered structure. The exosporium and endosporium were of equal thickness, the whole membrane being about 0·5 μ in thickness. Its double nature was also evident from the facts that both parts reacted differently to stains, and that a distinct curling of the membrane often took place; this is in agreement with the results of Thomson (8) on the megaspore membrane of the group. External to the membrane are two or three layers of cells in a disorganizing condition; the cells are often multinucleate and the nuclei react vigorously to stains suggestive of degenerating nuclei. These two layers form a tapetum, and several layers external to these are crushed by the growth of the developing megaspore (Text-fig. 9).

During the telophase of the last free nuclear division, while the daughter nuclei are being organized, the spindle is noticeably persistent, reacting readily to stains such as gentian violet and iron-alum-haematoxylin. Radiations are conspicuous at the poles. Wall-formation is initiated in the following way: Each daughter nucleus becomes gradually surrounded by radiations in the cytoplasm, which extend until they appear to reach those from neighbouring nuclei. Each daughter nucleus thus becomes connected to four, five, or six other nuclei (Text-fig. 10). These radiations result in the formation of a number of spindles, the radiations of which are generally coarser than those of the original spindle. The surrounding cytoplasm appears coarsely granular.



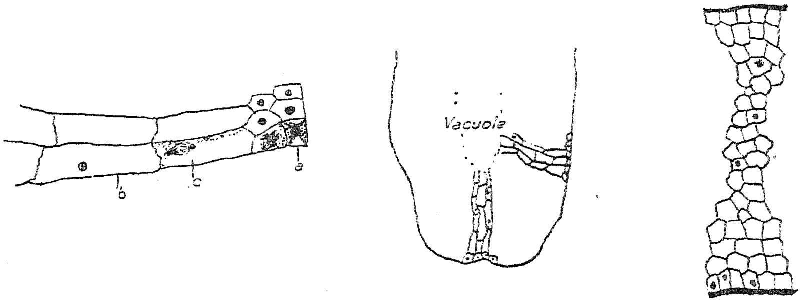
10. 11.

TEXT-FIGS. 10, 11. *Macrozamia Fraseri.* Development of ♀ gametophyte. 10. Wall-formation. 11. Parietal wall-formation.

The first indications of the cell plate appear on the mitotic spindle as thickenings which stain very readily, and are situated midway between the sister nuclei. These increase in amount, while the fibrillar appearance near the sister nuclei decreases. While this plate is forming granular thickenings appear on the other spindles. Each nucleus is then enclosed by a wall usually pentagonal or hexagonal in outline. The radiations disappear and the nuclei pass into a resting state (Text-fig. 11). This cell-plate formation proceeds throughout the parietal nuclei. Thus the free nuclei become partitioned off, but the wall does not attach them to the megaspore membrane, and they are open to the centre of the sac.

Division of these nuclei, followed by walls across the spindles, completes the formation of a small-celled layer, leaving again nuclei open to the centre. In this way, growth of the endosperm takes place until the sac is filled with tissue (Text-figs. 12, 13). After the first small-celled layer the cells are long and tubular, but later these become divided into smaller cells, the resulting cellular endosperm being a comparatively regular tissue (Text-fig. 14).

Though it is obvious that since centripetal growth of cells occurs there must be formed a definite median line from the abutting cells, no such line was found.



12. 13. 14.

TEXT-FIGS. 12–14. *Macrozamia Fraseri.* 12. Centripetal development of endosperm. *a*, small-celled external layer; *b*, long tubular cell; *c*, division of tubular cell. 13. Centripetal growth of endosperm. 14. Cellular endosperm.

The endosperm then in *Macrozamia Fraseri* has been shown to occur in the following stages:

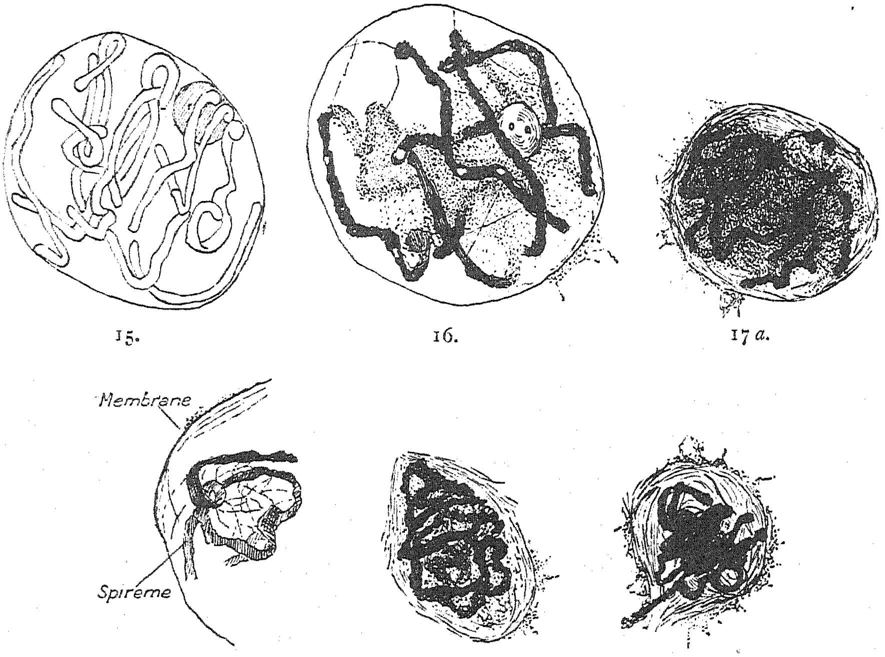
1. Free nuclear division,
2. Parietal wall-formation,
3. Centripetal development,

resulting in a comparatively regular tissue with no intercellular spaces.

THE MITOTIC DIVISION.

In the free nuclear division stage, the nuclei were found giving all the typical division figures in the one sac, and several hundreds were examined in each stage. In spite of the method of fixation the nuclei were apparently well preserved. Unfortunately, there was no possibility of any comparison with material fixed in the usual cytological fixatives. In any case a few of the observations made may be of general interest.

In the resting nuclei, one or two large nucleoli were present containing definite crystalline material. This is apparently dissolved away in prophase, though very often clear crystals were obtained in the nucleoli present in the spireme figures. The nucleoli generally appear in close contact with some portion of the spireme, and as the latter increases in chromatin, the nucleoli decrease and have disappeared by metaphase. The reticulum is very indefinite during rest, and all limits of the chromosome complex would appear to be lost. In telophase, however, a distinct spireme condition is passed through before rest, and as division begins the reticulum appears as a very long, fine, irregularly staining thread which gradually shortens and thickens as division proceeds. The thread in late prophase shows very clear vacuolate portions, which may be indications that the thread is longitudinally split. As uncut nuclei were only examined at this stage, details regarding this point were not obtained. The slit is clearly evident at metaphase. The thread does not appear homogeneous, but definite clear areas (not stainable in haematoxylin and safranin) define the limit of the chromosomes. As the shortening of the thread proceeds, it becomes segmented into twelve long chromosomes, a camera lucida drawing of which is shown in Text-fig. 15.

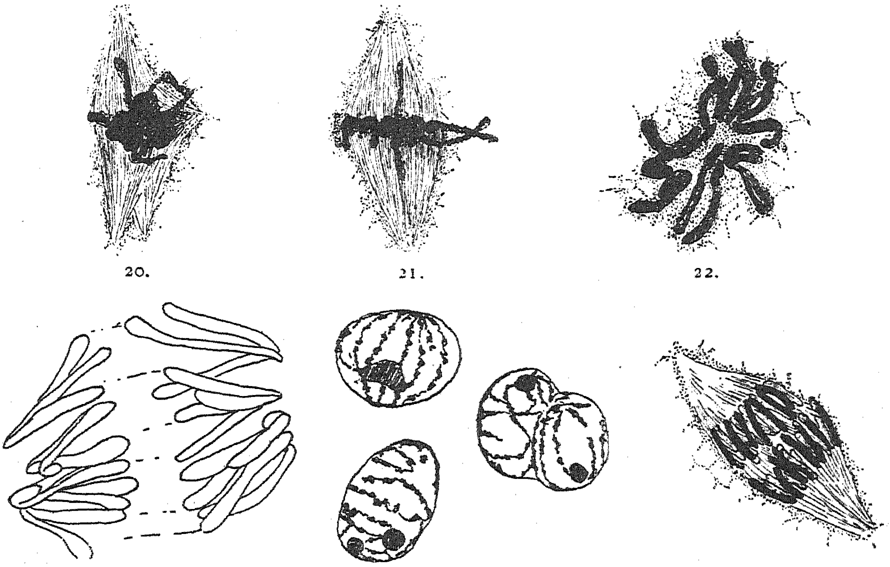


15. 16. 17 *a*.

17 *b*. 18. 19.

TEXT-FIGS. 15–19. *Macrozamia Fraseri*. 15. Spireme segmenting to form twelve chromosomes. 16, 17 *a*, and 17 *b*. Later stages showing further changes within the nuclear cavity. 18 and 19. Contraction of the nuclear area.

A change in form of the nucleus is often noticeable during the spireme changes. The nucleus changes from a spherical to an ellipsoidal form; the spireme is so placed within, that it winds parallel to the longer axis of the nucleus. Two other observations appear significant at this stage. The nuclear membrane stains irregularly and at some points very deeply with iron-alum-haematoxylin, and a denseness occurs in the nuclear sap, gradually increasing so that the limits of the spireme are no longer visible. Fine thread-like radiations appear just within the membrane. No changes in the external cytoplasm were noticeable. An attempt to represent this is shown in Text-figs. 16, 17 *a*, and 17 *b*. These changes cannot alone be attributed to fixation, since a gradual series can be traced from one nucleus to the next in one sac leading to definite spindles. Undoubtedly they represent in the fixed material a series of changes resulting in the achromatic figure.



TEXT-FIGS. 20–24 *b*. *Macrozamia Fraseri.* 20–23. Further stages in nuclear division; the anaphase shows twelve chromosomes. 24 *a*. Fusion of nuclei. 24 *b*. Abnormal division.

Reference to Text-figs. 18, 19, will show that a definite contraction of the nuclear area occurs; the radiations extend at first only at certain points into the surrounding cytoplasm. Gradually, however, the membrane is no longer apparent at any point and a multipolar figure is formed, the chromosomes being crowded together in the central region. The spindle becomes bipolar and the chromosomes lie at right angles to the spindle in the equatorial plate. Thus metaphase is reached (Text-figs. 20, 21).

Various polar views were obtained of this condition, showing the form and arrangement and number of the chromosomes. There were clearly twelve chomosomes; two or three were of a bent or hooked form, later evident in anaphase, but otherwise no distinction could be made between them. Their curled form did not allow measurement to be made, though in anaphase figures one chromosome seemed generally larger than the rest, showing a tendency to break up into two portions (Text-figs. 22, 23).

In one sac, many nuclei were larger than the others and of irregular form with lobed nucleoli. Together with these were some dividing, showing in anaphase a complement of chromosomes in excess of twelve. It is suggested that fusion between nuclei in free nuclear division may occur, and these may later undergo further division; such an abnormal condition is figured in Text-figs. 24 *a*, 24 *b*. The later stages in division appear without any particular point of interest. In telophase, the chromosomes form into a spireme which forms loops round the periphery of the newly forming membrane; the arrangement is very definite, each portion shows vacuolate areas, and connexions between neighbouring loops become established.

DISCUSSION.

The events described in this paper for the endosperm of *Macrozamia* indicate that in Cycads the development of this tissue conforms to the general type found throughout the Gymnosperms (excluding the Gnetales), as suggested by Coulter and Chamberlain. No attempt has been made to record a survey of the literature relating to the subject, but two accounts in particular figure stages which show a close parallel with those in *Macrozamia Fraseri*. These are found in papers by Lawson (9) on *Sequoia sempervirens* and Carothers (10) on *Ginkgo biloba*. Throughout the investigation of *Macrozamia* material, a gradual decrease in size of the nucleus was noticeable as growth of the endosperm increased. With this may be placed the fact that abnormal fusions and divisions of nuclei may occur. The endosperm in Gymnosperms is undoubtedly gametophytic as regards its chromosome content, and it is a tissue comparatively short-lived and functioning for the most part to provide nourishment for a developing embryo. It is not then unlikely that a gradual decrease in nuclear size should be found as the tissue matures (excluding the archegonia), or that abnormalities should occur where division is rapid.

In following the mitotic division, the chromosome number was found to be twelve. This number is seen to be in agreement with other records for the Cycads. It has been recorded for *Cycas revoluta*, Ishikawa (11), *Stangeria paradoxa*, *Dioon edule*, Chamberlain (4, 2), *Zamia Floridana*, *Ceratozamia mexicana*, Smith (12, 13). Also in two cases, *Cycas* and *Stangeria*, the diploid number has been counted as twenty-four. From the present records, this number twelve seems to be a surprisingly constant number throughout the whole group of Gymnosperms.

The formation of the spindle presented several points of interest. The preliminary elongation of the nucleus during prophase, the variability of the reaction of the membrane to stains, the increasing denseness in the nuclear sap, and the contraction of the nucleus are all regarded as relating to the formation of the achromatic figure. That the latter is, in its origin, entirely intranuclear seems quite evident, though there is no case in which its intranuclear form was seen as late as metaphase. Intranuclear metaphase figures are shown by Chamberlain in free nuclear division in the embryo, but from various accounts it does not seem a constant feature even in that tissue.

The elongation of the nucleus does suggest some possible external influence or merely a secondary result of changes occurring between the karyolymph and the cytoplasm. That centrosomes do occur in Cycads is evident from Chamberlain's and other figures of spermatogenesis, but their absence as indicated by staining reactions does not disprove their existence.

The radiations at the poles of the spindles are very similar to the astral radiations of centrosomes, and these may indicate the presence of otherwise invisible centrosomal bodies initiating repeated division, and possibly exerting some external influence on the nucleus. That changes occur between the karyolymph and the external cytoplasm is possible, since a definite contraction is seen in the nuclear area. This contraction suggests that some exosmosis occurs. Taken with this is the increase in staining capacity of the membrane. This is regarded as suggestive of its dissolution. In some cases a granular precipitate marked the position of the membrane. Changes obviously occur whatever may be the actual nature of the so-called membrane, resulting possibly in changes in permeability. Where karyolymph and cytoplasm meet some change occurs which gives in fixed material a remarkable denseness within the nucleus.

A similar contraction of the nuclear area has been recorded by Miss Nothnagel (14) in the pollen-mother divisions in *Allium*, and changes in intranuclear substance as the result of reactions between karyolymph and cytoplasm by Devisé (15) in the Larch microsporocytes. The suggestive paper of Cannon (16) on 'the nature of the centrosomal force', taken with recent work on micro-dissection, is of great interest. In its application to plant cells several difficulties present themselves, i.e. the absence of the centrosome and the presence of intranuclear spindles. These one supposes vanish on the acceptance that the essentials of the centrosomal force are present if the visible limits of such a body are absent. In the free nuclear divisions in *Macrozamia*, as indeed in subsequent divisions, the spindles lie always in the direction of the greater protoplasmic mass, and show a fairly constant length. Its limitation as suggested by Cannon would not be similar to those of a walled cell, but would be limited probably by the repellent forces between the poles and the chromosomes, between the two poles, and between each pole and those poles of other figures surrounding it. Whether the multipolar state of the developing spindle could be directly due to centrosomal action is difficult to say.

In free nuclear division, the dividing centrosomes, providing the oscillating bodies necessary for Cannon's theory, as they moved apart repelling one another, might interfere with other dividing centrosomes. Thus they would form at first a number of centres to which radiating streams of material would move before the final position of each centrosome was arrived at, and the bipolar condition attained. Obviously, it is not a point upon which a definite statement can be made.

SUMMARY.

1. The formation of the endosperm in *Macrozamia Fraseri* is described as proceeding in three stages: free nuclear division, parietal wall-formation, centripetal development.
2. The haploid chromosome number is found to be twelve.
3. The mitotic division of the endosperm nuclei is described.

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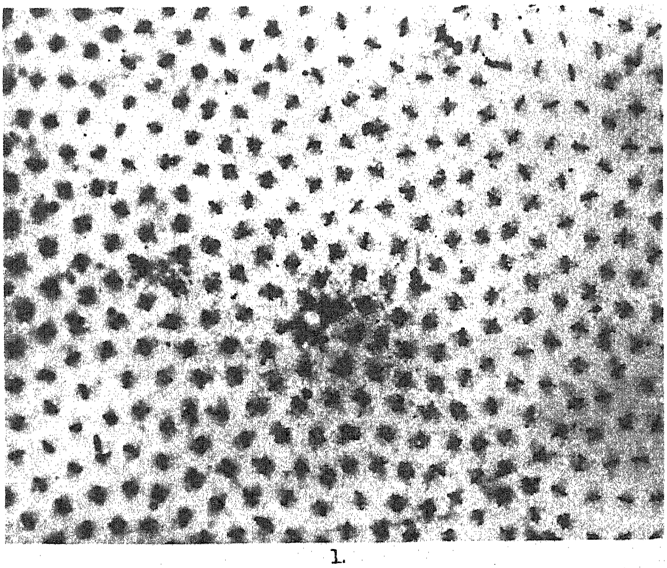
EXPLANATION OF PLATE X.

Illustrating Miss Light's paper on *Macrozamia Fraseri.*

Figs. 1–4 are microphotographs taken of portions of the Endosperm in the free nuclear stage.

Fig. 1. Numbers of dividing nuclei in metaphase, showing one polar view of chromosomes.
Fig. 2. Later stages in division, anaphase, and telophase.
Fig. 3. Parietal wall-formation beginning, granular thickenings on mitotic spindles.
Fig. 4. One nucleus from Fig. 2, enlarged. (× about 1,000).



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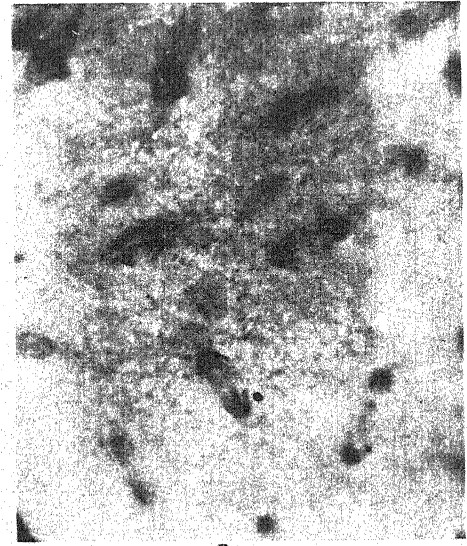

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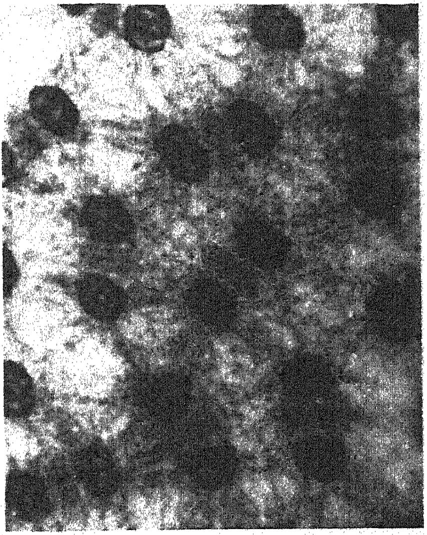

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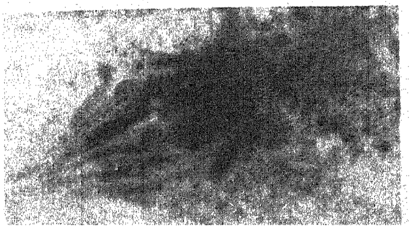

A Further Contribution to the Morphology and Physiology of the Genus Eidamia.

BY

A. S. HORNE, D.Sc.,

AND

G. HOWARD JONES, B.A.

From the Department of Plant Physiology and Pathology, Imperial College of Science and Technology, London.

With four Figures in the Text.

I. INTRODUCTION.

IN the course of an investigation of gooseberry disease in East Sussex it was found necessary by one of us (G. H. J.) to endeavour to isolate parasites from diseased gooseberry wood. Many different fungi were isolated in an impure condition, including one which produced a brown filmy growth in potato mush agar, which owed its appearance to the presence of large brown tuberculate spores. The original cultures were contaminated by bacteria and a species of *Fusarium*, but later pure cultures were obtained from these by the usual methods. This fungus, when examined, was observed to present an extraordinary resemblance in the method of branching and spore production to the genus *Monopodium* of Delacroix. A more thorough search, however, revealed the presence of a second aspergilliform type of sporophore which is a critical character of the genus *Eidamia* (Lindau). This type was of rare occurrence, and at first very difficult to find when known to be present in the cultures, but later it was found possible to render it quite obvious if the preparations were stained with cotton blue. Since the fungus does not appear to be identical with any species hitherto described, the authors regard it as a new species within the genus *Eidamia*, and propose the name of *Eidamia tuberculata*, having regard to the tuberculate macrospores.

[Annals of Botany, Vol. XXXVIII. No. CL. April, 1924.]

II. MORPHOLOGICAL CHARACTERS.

The fungus, when grown on potato mush agar at 25° C., forms a colourless mycelium consisting of repeatedly branched hyphae, usually 3–5 μ in diameter.

The macrospores are usually borne singly, though in two different ways. They may be situated on straight sporophores of variable length inserted laterally upon the main hyphae (Fig. 3), or they may be borne on sporophores which exhibit the peculiar monopodial branching which is so marked a feature of the genus *Monopodium* (Fig. 1, *b*, *d*). The macrospores are brown, tuberculate, thick walled, spherical or very slightly ovoid, and usually exhibit variations in size between 18 × 17 μ and 24 × 22 μ (Fig. 1, *c*). The spore-coat consists of a delicate intine and a thick brown warted extine approximately 1–1·5 μ thick. The spore contents are hyaline, and occasionally highly refractive globules are present.

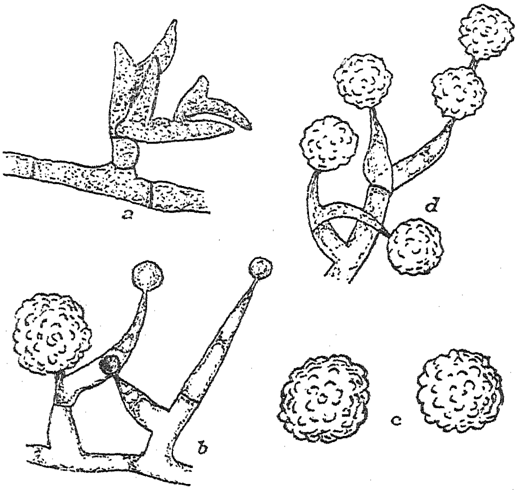

FIG. 1. *Eidamia tuberculata*. Macrospore stage. *a*, young branched conidiophore; *b*, older stage; *c*, macrospores; *d*, macrospores connected by isthmus (grown in agar).

The conidia (microspores) are borne typically upon conidiophores of the Aspergillus type (Fig. 2, *a*, *b*), but exceptionally the swollen head is absent and the sterigmata are grouped together on slightly swollen lateral hyphae, as originally noted by Bainier and Eidam (Fig. 2, *c*). The typical conidiophores are of variable length, 80–160 μ, and sometimes branched (Fig. 4 *a*). The apical head varies from 12 × 10 μ to 20 × 14 μ in size. The sterigmata are flask-shaped, 3–4 μ wide at the base and 4–6 μ long. The conidia, which are hyaline, spherical or ovoid, and 1·5–2 μ in diameter, are borne apically either in short chains or groups on the sterigmata.

The microspore fructifications exhibit a very close resemblance to those of *Eidamia acremonioides* (see H. and W., p. 395, Figs. 2, 3).

The following deviations from the typical morphological structure are sometimes present in cultures:

(*a*) The macrospores may be borne on sterigmata of a similar shape to, but larger than, those which bear microspores. In such cases the sporophore bears a single sterigma at its apex, or rarely two or more catenulate sterigmata, of which only the terminal one bears a macrospore (Fig. 3).

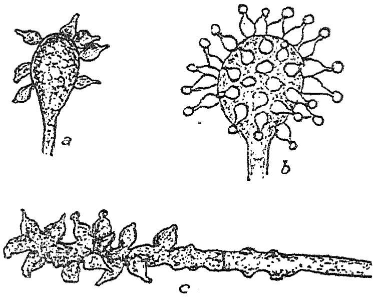

FIG. 2. *Eidamia tuberculata.* Microspore stage. *a*, young conidiophore of the aspergilliform type; *b*, older stage; *c*, sterigmata borne on a hypha.

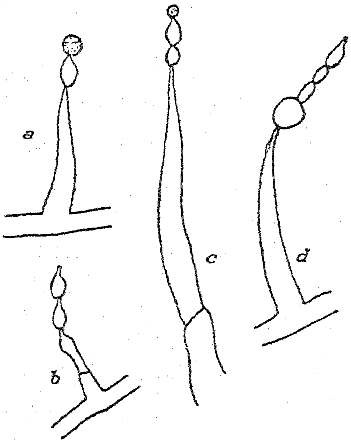

FIG. 3.

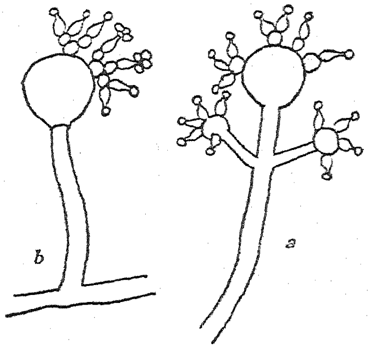

FIG. 4.

FIG. 3. *Eidamia tuberculata.* Macrospore stage. *a*, simple conidiophore terminated by a sterigma bearing a young macrospore; *b*, *c*, simple conidiophores bearing catenulate sterigmata; *d*, young macrospore bearing catenulate sterigmata.

FIG. 4. *Eidamia tuberculata.* Microspore stage (semi-diagrammatic). *a*, branched conidiophore of the aspergilliform type; *b*, sterigmata attached to spherical bodies arising from the globular head.

(*b*) A macrospore borne on a simple sporophore may terminate in a sterigma or catenulate sterigmata (Fig. 3, *d*).

(*c*) Sometimes a second macrospore occurs situated at the antical end of the first. When grown on agar without other nutrient two macrospores with a short connecting isthmus were occasionally observed (Fig. 1, *d*).

(*d*) A lateral hypha resembling a simple sporophore for the greater part of its length exhibits monopodial branching at its extremity.

(*e*) The swollen apex of the microsporophore may bear spherical protuberances to which the sterigmata are attached (Fig. 4, *a*, *b*).

(*f*) The swollen apex of the microsporophore may bear short hyphal branches, each terminating in a sterigma, in addition to sterigmata attached in the normal manner.

Most of these features were observed in cultures of *Eidamia tuberculata* in potato mush agar. Similar deviations from the typical structure were exhibited by *E. acremonioides* when grown in this medium.

Eidamia tuberculata, n. sp.

Mycelium hyalinum vel brunneum, septatum, ramosum, plerumque 3–5 μ latum; conidiophora erecta, hyalina, simplicia vel ramosa, plerumque 80–160 μ longa, ad apices turgida, 12×10–$20 \times 14\,\mu$; sterigmata lageniformia, ad basim 3–5 μ lata, 4–6 μ longa, tum ad apicem turgidum conidiophori tum ad hypham ipsam deposita; conidia (microsporae) hyalina sphaerica, 1·5–5 μ diam. vel subovoidea $4 \times 5\,\mu$; macrosporophora simplicia $28 \times 48\,\mu$ longa vel monopodialiter ramosa ramis hyalinis vel brunneis; sterigmata (saepe absenta) microsporophoris similia sed longiora; macrosporae brunneae, tuberculatae, pachydermaticae, sphaericae vel subovoideae, 18×17–$24 \times 22\,\mu$, solitariae vel binae catenularii formantis.

E. acremonioides similis sed macrosporis tuberculatis, mycelio copiosissime et microsporis rarioribus differt.[1]

III. PHYSIOLOGICAL CHARACTERS.

A. *Growth on Nutrient Media* (*Plate Cultures*).

Series of parallel cultures of *E. acremonioides* and *E. tuberculata* were set up using Crabill's medium, potato mush agar, and potato extract agar at three different temperatures (20°, 25°, and 30° C.). After seven days the following results were obtained:

At 30° C. no growth in both cases, except that *E. tuberculata* produced a small colony of dense texture on potato mush agar.

At 25° C. a well-marked growth difference between the two species when grown in Crabill's medium and potato extract agar was apparent, *E. acremonioides* producing growth of a feeble character, whereas in the case of

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[1] The authors are indebted to Mr. J. Ramsbottom, of the British Museum (Natural History), for this description.

E. tuberculata a dense mycelial felt associated with marked sporulation was formed. Both species produce well-marked growth in potato mush agar, though the former distinction, notably in relation to mycelial growth, which is the predominating feature in *E. tuberculata*, is still obvious. In *E. acremonioides* the colour of the culture is tawny olive to Saccardo's umber, whereas in *E. tuberculata* sepia predominates.

At 20° C. there was again a well-marked difference between the two species.

(*a*) *Crabill's medium.* In the case of *E. acremonioides* the growth was of a filmy spreading character, whereas that of *E. tuberculata* was more compact and exhibited fluffy aerial mycelium.

(*b*) *Potato extract agar.* The differences are of a similar character. The growth of *E. tuberculata* is more compact, and the colony possesses a more strongly defined margin than is evident in Crabill at this temperature.

(*c*) *Potato mush agar. E. tuberculata* exhibits a dense mycelial felt somewhat similar to that formed at 25° C., but the colour in the substratum is slightly more intense and the sporing more prolific. The colour of sepia predominates. In *E. acremonioides* the growth is less pronounced than at 25° C. and sporing less prolific. Saccardo's umber is the predominating colour.

From these results it is evident that *E. tuberculata* is a more vigorous fungus than *E. acremonioides.* A similar conclusion was reached from observations on parallel series of cultures in all three media kept at room temperature (variable).

Very little difference in the amount and nature of the growth and colouring present in parallel cultures on these media developed in the light and dark respectively.

With regard to temperature relations, Horne and Williamson give 20° C. as the approximate optimum temperature for *E. acremonioides.* This is in agreement with the results obtained here for Crabill's medium and potato extract agar. In the case of *E. tuberculata* little difference in the growth produced both on Crabill's medium and potato mush agar at 20° and 25° C. could be detected, whereas in potato extract agar more growth occurred at 25° C.

B. *Growth in Carbohydrate and Protein.*

1. *Starch.*

Petri dishes containing Crabill's medium were inoculated with *E. acremonioides* and *E. tuberculata* respectively, and kept for seven days at 20° C. In the case of *E. acremonioides* the fungus made little growth, while under the same conditions *E. tuberculata* had almost covered the plate. On flooding the plate with iodine solution the whole of the medium containing *E. acremonioides* remained blue, but in the case of *E. tuberculata* a circular area (lenticular in cross-section) 5·5 mm. in diameter remained colourless, indicating complete hydrolysis: this area was surrounded by a zone, 4 to 8 mm. in width, of a reddish colour indicating the presence of erythrodextrin. Owing to the difference in the amount of mycelial growth produced by the species the results are not strictly comparable. When areas of equal mycelial growth were compared, again *E. acremonioides* showed no sign of starch hydrolysis, whereas *E. tuberculata* gave evidence of a vigorous reaction—no evidence of starch hydrolysis by *E. acremonioides* was obtained by Horne and Williamson when this species was grown in solutions containing starch (H. and W., p. 405).

2. *Glucose.* (H. and W., pp. 401, 402.)

Flasks containing a 4 per cent. concentration of glucose were inoculated with *E. acremonioides* and *E. tuberculata* and kept for five weeks at 20° C.

Result:—*E. acremonioides.* Very feeble growth, involution forms predominate: normal macrospores present: microspore stage absent.

E. tuberculata. Thin surface growth with abundant gelatinous growth in the liquid: mycelium closely packed with highly refractive bodies; macrospores in moderate abundance, smaller and darker than those occurring in peptone and peptone-glucose, macrosporophores usually simple: the monopodial type of branching not typically developed: microspore stage not observed. Liquid neutral to litmus (as control).

3. *Sucrose.* (H. and W., p. 402.)

Flasks containing sucrose in 4 per cent. concentration were inoculated and kept for five weeks at 2c° C.

Result:—*E. acremonioides.* Very feeble growth. No inversion.

E. tuberculata. Somewhat cloudy submerged growth. Inversion (strong precipitate with Fehling).

4. *Peptone.* (H. and W., p. 407).

Flasks containing a 2 per cent. concentration of peptone were inoculated and kept at 20° C. for five weeks.

Result:—*E. acremonioides.* A thin superficial mycelial growth: olive brown, closely septate hyphae present: numerous macrospores of the usual type present: microspore stage common: conidia almost invariably in groups. Liquid neutral to litmus (as control). Ammonia not evolved.

E. tuberculata. Dense surface growth: numerous macrospores of normal type present: the first macrospore sometimes bears a second at its antical end: involution spore types present: microspore stage not observed. Liquid slightly alkaline. Ammonia liberated.

5. *Peptone and Glucose.* (H. and W., p. 408.)

Flasks containing peptone in 2 per cent. and glucose in 4 per cent. concentration were inoculated and kept for five weeks at 20° C.

Result:—*E. acremonioides.* Moderate superficial growth. Very numerous macrospores which are occasionally borne on relatively large flask-shaped sterigmata or swollen end-cells: typical microspore stage not observed. Liquid slightly acid to litmus (as control). Ammonia not evolved.

E. tuberculata. Prolific growth: macrospores of normal type very numerous: microspore stage not observed. Liquid slightly alkaline. Ammonia evolved.

IV. THE IDENTITY OF *MONOPODIUM UREDOPSIS*, DELACROIX, AND *EIDAMIA ACREMONIOIDES*, HARZ.

In connexion with a survey of the genus *Eidamia* Horne and Williamson came to the conclusion that the identity of *Monopodium uredopsis*, Delacroix, and *Eidamia acremonioides* would be ultimately established, since *Monopodium uredopsis*, according to Delacroix's description of the fungus, appears to differ from *Eidamia acremonioides* only in the absence of a microspore stage—the monopodial method of branching of the macrosporophore and the macrospores themselves exhibiting extraordinary similarity in the two cases. Since the publication of Horne and Williamson's work, the present authors have obtained a culture of *Monopodium uredopsis* from the Centraalbureau voor Schimmelcultures, Baarn. The medium upon which the fungus was growing when it was received consisted of a nutrient agar upon which a few grains of wheat had been placed. The culture was two months old when examined. In its filmy appearance and colour (due to the macrospores) the growth in the tube presented a strong resemblance to *Eidamia acremonioides.* An equally striking resemblance was presented in morphological characters; in fact, no general deviation in the method of branching, form of conidiophore, and shape and dimensions of the macrospores could be detected. Nevertheless, although a thorough search was made, no trace of a microspore stage was observed.

Subcultures on potato mush agar, using inoculant from the Baarn culture, were then made. At the end of ten days these cultures were examined in detail, and a microspore stage was discovered which resembled the aspergilliform conidial stage obtaining in *E. acremonioides* in every particular.

The resemblance will be made perfectly clear if the following data obtained from the growth in potato mush agar are compared with those given for *Eidamia acremonioides* (H. and W., p. 394).

Macrospore conidiophores simple, reaching 100 μ long, branched in a monopodial manner; branches hyaline or brown, usually about 28 μ, but sometimes 48 μ long. Macrospores brown with smooth wall, obovoid, 16–36 × 16–26 μ with extreme dimensions 16 μ in diam. and 36 × 25 μ.

Microsporophore hyaline, often exceeding 160 μ long; aspergilliform heads average about 20 × 18 μ. Microspores also borne on the mycelium. Sterigmata flask-shaped, 4 × 5–6 μ. Conidia chiefly in groups and spherical, averaging 1·5–3 μ in diam. and occasionally larger (4 × 5 μ).

The chief tests which proved useful in establishing the physiological relations between the species of *Eidamia* have been employed in the case of *Monopodium uredopsis*; as a result the reactions exhibited by this fungus towards starch, sugar, peptone, &c., prove to be the same as those given by *Eidamia acremonioides*.

The following facts may be regarded as established:

1. The characters presented by the Baarn growth of *Monopodium uredopsis* agree with those described for the fungus by Delacroix.
2. The characters presented by the Baarn growth of *Eidamia acremonioides* agree with those described for the fungus by Harz.
3. The fungus present in the culture of *Eidamia acremonioides* is identical with that in the culture of *Monopodium uredopsis*.

Langloisula macrospora (see slides by Smith and Pethybridge in the British Museum of Natural History) is morphologically indistinguishable from *Monopodium uredopsis*. In the light of the facts explained above it seems quite certain that this fungus also is actually *Eidamia acremonioides*, the microspore stage having been overlooked through obtaining growths in media unfavourable to microspore development. This fungus was erroneously referred to the genus *Langloisula*, being quite unlike the type species *Langloisula spinosa*, Ellis and Everhart.

V. DISCUSSION.

Morphologically *E. tuberculata* closely resembles *E. acremonioides*, the outstanding differences being related to the structure of the macrospores—tuberculate and smooth respectively. In both species the macrospores are coloured brown and the microspores are situated either in groups or chains on sterigmata borne on the swollen apex of the conidiophore, features wherein they differ from *E. catenulata* and *E. viridescens*, where the macrospores are hyaline and the sterigmata are not usually seated on an aspergilliform head.

In physiological characters *E. tuberculata* and *E. acremonioides* exhibit several well-marked differences which are tabulated on p. 359.

Since the results given in the table for *E. acremonioides* agree very closely with those obtained by Horne and Williamson (H. and W., pp. 402, 406, 407, 408) for the same fungus, the data given for *E. tuberculata* may be fairly used for comparative purposes. In spite of the close morphological resemblance exhibited by the two species, the difference in their reactions towards carbohydrate and protein is considerable in fact physiologically. *E. tuberculata*—except in the power of fermenting sugar in peptone-glucose solutions—is in closer agreement with *E. catenulata* and *E. viridescens*, species which differ morphologically from it more widely.

Medium.	*E. tuberculata.*	*E. acremonioides.*
Starch	well-marked hydrolysis	not hydrolysed
Cane sugar	inversion (strong reaction)	not inverted
Glucose	moderate growth	very feeble growth
Peptone and glucose	prolific growth liquid rendered alkaline ammonia liberated	much growth liquid acid ammonia not evolved
Peptone	dense growth liquid rendered alkaline ammonia liberated	feeble growth liquid neutral ammonia not evolved
Protein hydrolysis (other than peptone)	pronounced	feeble growth
Malic acid	feeble growth	no growth

Although the hydrogen-ion concentration limits have not been determined a range somewhat similar to that obtained for *E. acremonioides* (H. and W., pp. 409, 410) is indicated from its behaviour in malic acid.

Owing to the relative infrequency of the occurrence of the microspore stage in cultural media, the comparative rarity of this stage in many media where it is present, and the fact that it is easily overlooked in unstained preparations, great care should be exercised in the diagnosis of fungi having a general resemblance to *Monopodium* (macrospore stage of *Eidamia acremonioides*). The importance of realizing that in certain media the species of *Eidamia* do not produce microspores has been emphasized in the section of this paper specially concerned with *Monopodium.*

SUMMARY.

1. A new species of *Eidamia*, *E. tuberculata*, is described which differs from *E. acremonioides* in possessing tuberculate macrospores and in its ability to hydrolyse starch, invert cane sugar, and decompose peptone.
2. The identity of *Monopodium uredopsis*, Delacroix, and *Eidamia acremonioides*, Harz, is established.

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A Preliminary Account of the Chromosomes and Chromosome Behaviour in the Salicaceae.

BY

KATHLEEN B. BLACKBURN

AND

J. W. HESLOP HARRISON.

With eleven Figures in the Text.

THE work outlined in the present paper was undertaken with several ideas in view, the most important of which were as follows:

1. It is well known that many of the species or microgenes of the Salicaceae resemble the Rosae in their excessive polymorphism. In seeking for a cytological explanation of the situation in the Roses, we discovered that most of them possessed a peculiar chromosome complement which rendered a perfect heterotype division an impossibility. In addition the succeeding stages of microspore development showed other characteristic abnormalities developed. Comparing this unusual behaviour with that of recognized hybrids during the meiotic phase, we concluded that hybridity was the inciting cause. Since *Salix* exhibits the same variability, we deemed it possible that the same root cause, hybridity, accounted for its peculiarities; thus our problem led us to study meiosis in the Salicaceae in order to determine whether it was of the normal or of the Rose type.

2. Recent work by Heribert-Nilsson has shown that, in the phenhybrids between pairs of *Salix* species exhibiting opposed and readily recognized characters such as leaf breadth, &c., the inheritance of such proceeds on Mendelian lines. If the chromosomes form the mechanical basis of Mendelian phenomena, then in the micro- and megaspore formation of *Salix* hybrids many or all of the chromosomes should encounter partners in the stages leading to the reduction division.

3. As the Salicaceae are dioecious we had hopes of demonstrating the existence of so-called sex-chromosomes in thier two genera.

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4. Lastly, a comparative study of the chromosome complements of the species of *Populus* and *Salix* was clearly of paramount importance in ascertaining their relations to each other, and thereby enabling some light to be cast on the systematic obscurity surrounding many of the species.

MATERIAL AND METHODS.

Most of our material was collected and determined by ourselves, but for much valuable assistance with rarer species we have to thank the Director of Kew Gardens, the Regius Keeper of the Edinburgh Botanic Gardens, and the Director of the Cambridge Botanic Gardens. We are also greatly indebted to Professor M. C. Potter for providing every possible facility for the carrying out of this research.

Generally, the methods adopted were those set out in our paper on the Roses, but modifications in detail were necessary owing to the practical impossibility of cutting satisfactory sections from complete catkins in most species. In cases presenting difficulties, these arose from the dense vestiture of hairs on the bracts and axis, which rendered it essential to dissect out the individual stamens or carpels previous to fixation.

All the figures were drawn with a camera lucida and reduced to a magnification of 3,300, except Figs. 4, 9, and 11, of which the magnification is 3,000.

Populus.

The aspen (*Populus tremula*, L.) was first submitted to examination, and the male catkins formed ideal material for cytological work. Every feature in the meiotic phase, from the resting-stage prior to synapsis, right through to the perfect microspore, proved diagrammatic in its beauty and simplicity. At both the heterotype and homotype divisions the number of chromosomes found in horizontal plates was constantly nineteen. These were of unequal dimensions; nine were small ones of more or less uniform size; nine others, larger than these, formed a graded series beginning with a member of just a little greater volume than the individual of the first group, and ending with one more than four times its volume. Lastly, there was a single chromosome, obviously compound in structure, nearly always appearing in a flat plate as four-lobed, equalling in volume, if not exceeding, that of any two of the other eighteen. Rarely this chromosome displayed one of its lobes almost detached from the others (see Fig. 1, *b*). Furthermore, six of the second series were at times obscurely compound in structure. In heterotype anaphases, when both of the daughter plates could be traced and counted, one of these smaller chromosomes appeared to be homologous with a spherical non-lobed one of distinctly smaller volume than itself (see Fig. 1, *a*, p_1, p_2). These unequally paired chromosomes have been detected in good profile views of the heterotype division (Fig. 1, *c*) as well as at the diakinetic stage.

For these results we rely on many perfect figures, and from them we are entitled to conclude (1) that the chromosomes here are entities with characteristics sufficiently well marked to enable one to identify them; (2) that some evidence exists of the presence of heterochromosomes, possibly sex-determining in their import, in the male of *Populus tremula.* In the female none of our preparations showed significant stages, but, fortunately enough, in both male and female we were able to make somatic counts, and these in every case were thirty-eight.

Populus nigra, Linn. Here, in the male, conditions were much the same as in the aspen, and the haploid number was nineteen, and the diploid thirty-eight.

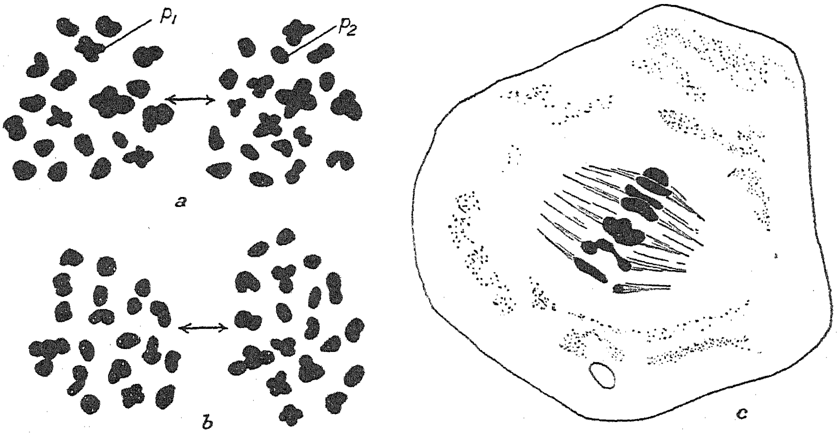


FIG. 1. *Populus tremula.* Heterotype division. *a* and *b*. Pairs of anaphase plates. *c*. Profile view of the metaphase.

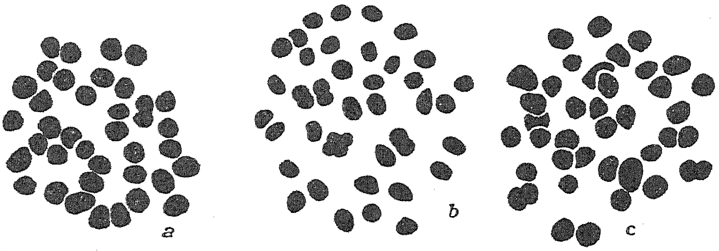
Populus balsamifera, Linn. No material presenting critical stages in meiosis has been studied in the balsam poplar, but that dealt with yielded very good somatic metaphase plates affording evidence that, in all probability, the somatic number was seventy-six, in which case the species would appear to be tetraploid.

THE *SALIX* SPECIES.

(a) *Section Pentandrae.*

The Pentandrae studied included two forms: *Salix pentandra*, Linn., of European origin, and *S. lucida*, Mühl., a native of America. Of these, both sexes were examined in the former and only the male in the latter. So similar were the males of *S. pentandra* and *S. lucida* in pollen development that, despite the obvious differences between the two plants, one is tempted to see in them simply local races of one and the same species rather than a pair of representative species—more especially when the *angustifolia* form of the European plant is considered.

In both the haploid chromosome yield is thirty-eight, and, in addition, they agree in cell and chromosome size (cf. Fig. 2, *a* and *c*). Just as in *Populus tremula*, certain chromosomes seem lobed or complex, but in these two plants only three at the most are so built up, for nearly all are of equal size. Nevertheless, the evidence gained from their study suffices to support views as to the individuality and continuity of the chromosomes. The single heterotype plate detected in *S. pentandra* var. *angustifolia*, female (Fig. 2, *b*), was in entire agreement with the heterotype plates of the male, both in the actual chromosome count and in individual characteristics.



S.pentandra ♂ S.pentandra var angustifolia ♀ S.lucida ♂

FIG. 2. *Section Pentandrae.* *a–c.* Heterotype metaphases.

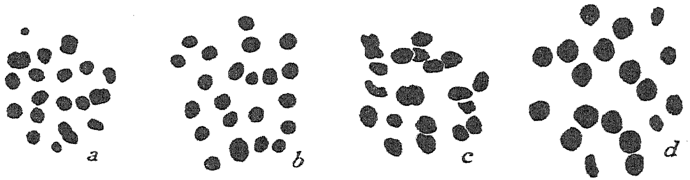
Somatic counts of over seventy chromosomes in *Salix lucida* and *S. pentandra* males, as well as in the female of the *angustifolia* form of the latter, confirmed our opinion that reduction was normal in these two species.

(b) *Section Amygdalinae.*

Salix triandra, Linn. The first supply of the Almond-leaved Willow to be considered came from Bedfordshire, and in this case, as in the aspen, the counts made gave nineteen as the haploid number (Fig. 3, *a*). However, both nuclear size and chromatin mass were inferior to those of either *Populus tremula* or of *Salix pentandra*. Four largish chromosomes were subequal and more or less markedly compound, thirteen were roughly spherical in shape and fairly even in size, while two were quite small, and one in particular pronouncedly so (Fig. 3, *a*). The number nineteen could be made out with equal facility in the many homotype plates available. Confirmatory of this number were observations of thirty-eight chromosomes made out in a series of somatic cells.

Our other material was provided by Kew, and quite naturally we anticipated that it would agree with the former. Such agreement, however, was not found, for in every heterotype plate examined, as well as in homotype metaphases, the number of chromosomes was twenty-two instead of the expected nineteen. The average chromosome size was somewhat greater than in the Bedfordshire material, and in the group of plates submitted to critical examination the individual chromosomes seemed to be placed farther apart (see Fig. 3, *b*). From a prolonged consideration of the preparations we were driven to the conclusion that this second type of *Salix triandra* arises from the first through a break in one or more compound chromosomes homologous with those referred to in our discussion of *Populus tremula.*

In harmony with this second haploid number for the species were somatic counts made in the same material which gave totals of certainly more than forty.



S. triandra S. triandra S. daphnoides S. purpurea

FIG. 3. *a–d.* Heterotype metaphase plates.

(c) *Section Fragiles.*

Salix fragilis, Linn., and its variety *Basfordiana.* In this species both the type and that supplied by Kew as var. *Basfordiana* were investigated.

During the preparatory stages leading to the heterotype division, and at the assembly of the bivalents for that stage, the chromosome number, general appearance, and behaviour differ but little from what was described for *S. pentandra.* Only in a slight sluggishness of the individual chromosomes in reaching the equatorial plate can any difference be made out. However, after that point, irregularities in procedure arise both in the type plant and in the variety; some chromosomes lag on the plate, others at various positions on the spindle, thereby giving it a ragged appearance, whilst yet others tend to wander out into the cytoplasm (Fig. 4, *c*).

Thus, at the interkinesis, micronuclei, recalling those encountered in *Rosa*, may arise with consequent deficiencies in the reckoning at the homotype division (Fig. 4, *d*). Only occasionally, therefore, can the full thirty-eight be made out at that period (see, however, Fig. 4, *e*). Compared with *Rosa*, the abnormalities are insignificant, and, except that micronuclei pass into the sphere of influence of the major nuclei, tetrad and pollen formation are not seriously disturbed (Fig. 4, *f*).

In this species we succeeded in securing good counts in somatic prophases when, as nearly as possible, seventy-six chromosomes could be seen (Fig. 4, *b*).

(d) *Section Albae.*

Salix alba, Linn. This species behaves in quite an ordinary manner at each step in meiosis, and therefore resembles *Salix lucida* and *S. pentandra* rather than *S. fragilis*, with which one is more inclined to associate it. Again, the reduced chromosome complement is thirty-eight (see Fig. 5), and the somatic count made from the same material seventy-six.

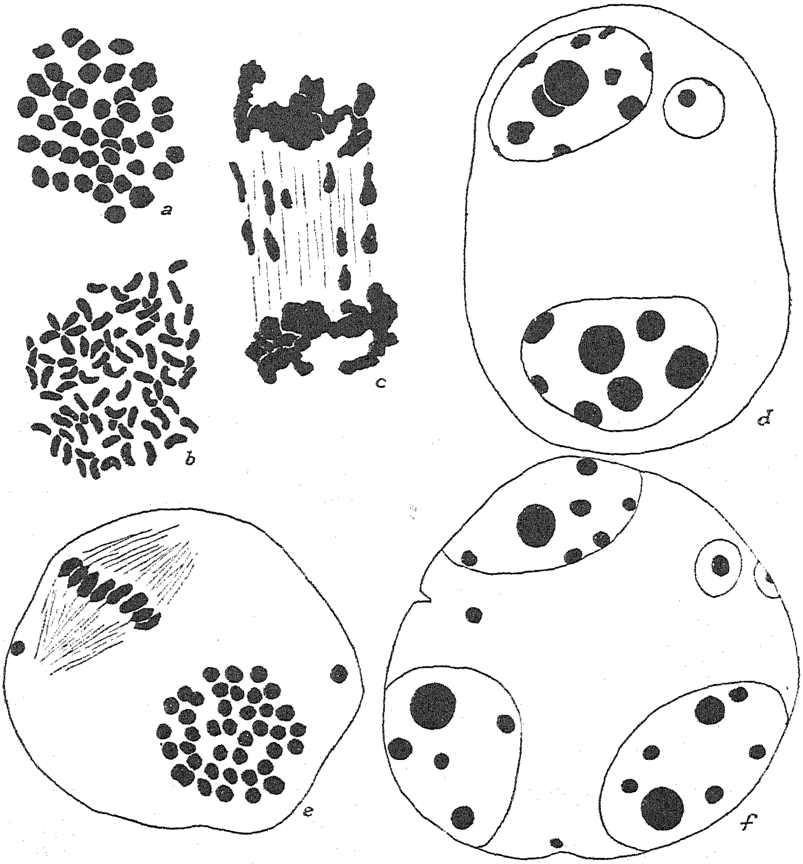


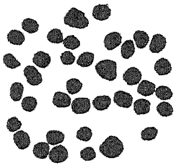
FIG. 4. *S. fragilis*. *a–f*. Meiotic phases and somatic plate at *b*.

(e) *Section Pruinosae.*

Salix daphnoides, Vill. Once more we are concerned with a species easily differentiated from the other Salices, but from the standpoint of its cytology presenting no unusual features in microspore formation. When we compared it point by point with our Bedford *S. triandra* little divergence was discoverable, although in *S. daphnoides* the average chromosome dimension seemed just a little greater (cf. Fig. 3, *a* and *c*). *Salix daphnoides*, therefore, falls in line with the diploid *Salix* species and possesses a reduced complement of nineteen; with this figure the somatic count of thirty-eight, made in very many sections, is in strict accord.

(f) *Section Purpureae.*

Salix purpurea, Linn. This species, likewise well characterized, also exhibits nineteen as its reduced chromosome number. Concerning its pollen formation little need be said, for the various stages leading to the appearance of the ripe pollen grain proceed with perfect smoothness.



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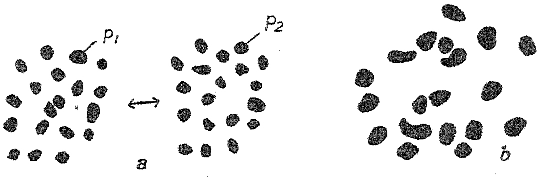

6.

FIG. 5. *S. alba.* Heterotype metaphase.
FIG. 6. *S. viminalis.* Heterotype division. *a.* Anaphase plates ♂. *b.* Metaphase ♀.

Preparations showing the different steps during meiosis cannot be distinguished from parallel ones taken from *S. daphnoides* (cf. Fig. 3, *c* and *d*).

Our preparations revealed no stages in the female beyond the synaptic knot, but in cells in the carpel wall this sex gave somatic determinations certainly over thirty-four but below forty—a figure agreeing with similar counts made in the male.

(g) *Section Viminales.*

Salix viminalis, Linn. Again we meet a plant the specific characters of which are such as lead to its easy determination.

Satisfactory preparations were made from both sexes, and in each case the haploid chromosome value of nineteen was determined (Fig. 6). Although pollen development throughout appeared quite typical, many interesting observations peculiar to the species were made, the most striking of which were the smallness of the individual chromosomes and their evenness in size, two at the most, and these only to a slight degree, being larger than the rest. In heterotypical anaphases, in the male material, where both daughter plates could be examined, a very slight disparity was noticeable between a member of this pair and its homologue (Fig. 6, *a*, p_1, p_2).

In both sexes the somatic count harmonized with the preceding observations, and was made out to be thirty-eight.

(h) *Section Capreae.*

This group, as far as its British representatives are concerned, comprises within its limits three species, *Salix Caprea*, *S. cinerea*, and *S. aurita*. So closely are these three forms allied that many workers, both here and abroad, look upon them as forming one Protean species, whilst others, prepared to concede that *S. aurita* is a valid species, regard the other two as varieties of one species. However, the striations of the old wood are enough to enable one to separate *S. Caprea* and *S. cinerea* with the utmost facility, even independently of other obvious differences seen best when the plants are submitted to careful study in the field.

Salix aurita, Linn. Material for the study of this species was derived from several sources, but that originating from Waldridge Fell, Co. Durham, provided our best preparations.

The stages leading to the heterotype division are very typical, and the metaphase plates reveal thirty-eight clear, well-spaced chromosomes (see Fig. 7, *a*). Of these one is much larger than the others, which show differences in size similar to those mentioned in the case of *Populus tremula*. The anaphases of this division show two perfectly even plates of separating chromosomes.

While the stages subsequent to pollen formation may be quite normal, very frequently serious disturbances appear in the homotype division. For instance, one anther loculus, in addition to containing typical homotype metaphases, displayed pollen mother-cells provided with tripolar spindles as well as irregular metaphase plates. Moreover, other homotype figures showed wandering chromosomes and, consequently, plates with chromosome numbers varying between twenty-one and twenty-nine.

Similarly, the amount of good pollen in the various anthers could be anything between 8 per cent. and 80 per cent.

That the disturbing factor in these abnormal cases is not irregularity in reduction is demonstrated by an excellent somatic plate which yielded an absolutely certain count of seventy-six chromosomes—a number also occurring in good somatic plates of the female.

As *Salix aurita* appears to be more or less apomictical, these abnormalities are no doubt connected with that curious phenomenon.

Salix cinerea, Linn. Like its ally, just considered, *S. cinerea* possesses as its haploid number thirty-eight chromosomes, and since its somatic complement, in both male and female, was seventy-six, as nearly as one could determine, reduction occurs in the ordinary fashion. Moreover, in accordance with anticipation, its homotype plates show thirty-eight chromosomes likewise (see Fig. 7, *b*, *c*, and *d*).

Curiously enough, none of the peculiarities developed in *Salix aurita* are manifested in this case, and this may be correlated with the fact that, although *S. cinerea* is partially parthenocarpic, it is not apomictical like *S. aurita*.

Salix Caprea, Linn. In this species our slides were made from material originating in several stations in Durham, Northumberland, and Bedfordshire, and both sexes were represented. However, in addition to this material definitely male or female, attention was directed to preparations made from a Birtley specimen of the so-called *metamorphosans* variety. This bush, although fundamentally a male, exhibits florets forming a complete transition between those purely male and those the femaleness of which is equally unchallengeable. It is to be noted, however, that examples falling into the latter category are very rare. In microspore development this plant provided no anomalies, nor was the pollen itself less perfect or potent than that taken from ordinary male plants. Its haploid chromosome number was nineteen, and the chromosomes themselves were spherical in shape and practically equal in size (Fig. 7, *f*). Similarly, the other bushes, whether male or female, from widely separated localities, gave the same haploid count of nineteen. Further, the individual chromosomes were the exact replicas of those seen in the intersex. On the average, these were about double the size of those of *S. aurita* (see Fig. 7, *e*).

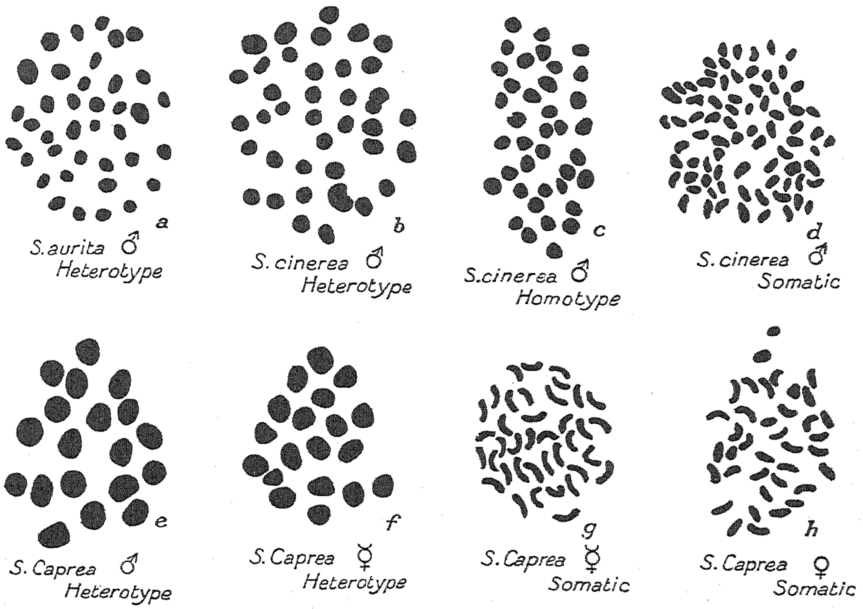

FIG. 7. *S. aurita, S. cinerea,* and *S. Caprea.* *a–h.* Metaphase plates.

In both male and female somatic cells, as well as in tissues of the intersex, no matter where placed, the somatic number was clearly thirty-eight (see Fig. 7, *g* and *h*).

(i) *Section Argenteae.*

Salix repens, Linn. Here we meet once more a species not likely to be confused with any of its allies, so sharply is it marked off morphologically, as well as in its habitats and time of flowering, from its congeners.

The material studied by us was secured from a colony growing amongst heather on Birtley Fell, Co. Durham, far removed from any other species of willow. In making our preparations we found it exceedingly difficult to find the exact times at which the reduction divisions were proceeding, so that, somewhat paradoxically, only in the female have we been able to examine a complete series. This difficulty we assign to the long-drawn-out period covered by the meiotic phenomena in the plant—a development no doubt necessitated by the extremely exposed nature of its chosen habitats.

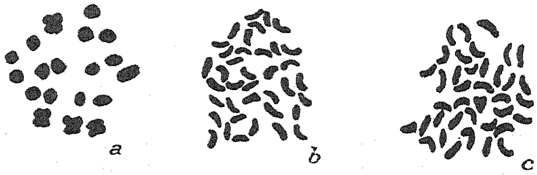

FIG. 8. *a* and *b*. *S. repens* ♀. Heterotype and somatic metaphases. *c*. *S. myrsinites*. Somatic plate.

In both sexes the stages of meiosis observed were quite normal and, as far as they went in the male, and completely in the female, might well have been provided by any ordinary phanerogam. The haploid chromosome number was nineteen, so that the species takes its place as a regular diploid member of the orthoploid series developed in the Salicaceae on the base number nineteen. Thus it falls in line with counts actually made for *Populus tremula*, *P. nigra*, *Salix purpurea*, *S. viminalis*, *S. daphnoides*, and *S. Caprea*, and with that deduced, as will be seen later, for *S. lanata* and *S. myrsinites*.

In cell size but little difference could be detected between it and *S. Caprea*, although in chromosome shape, but not in number, a closer approximation to *S. aurita* was manifested. However, the resemblance to *Populus tremula* in every respect was still more striking.

In the female four chromosomes, all markedly four-lobed and equal in size, were decidedly larger than the rest; a fifth, less than these and not four-lobed, was intermediate in size, whilst the other thirteen, apparently simple in structure, were practically equal, whether seen in polar view, in profile, or obliquely (see Fig. 8, *a*).

As we expected, the somatic number totalled thirty-eight, thereby proving that reduction in *Salix repens* is quite typical (see Fig. 8, *b*).

(j) *Section Myrtosalix*, Kerner.

Salix myrsinites, Linn. Although we obtained our supplies of *Salix myrsinites* from no less than three sources (Sweden, Kew, and Edinburgh), so great was the check on the growth and development of the catkins during transit to us] that no useful meiotic stages were to be discovered in our preparations. However, the somatic mitoses were so clear that we had no difficulty in counting thirty-eight chromosomes (see Fig. 8, *c*). Hence, almost certainly, its haploid number is nineteen, which brings it into harmony with the orthoploid series of the family.

(k) *Section Phylicifoliae.*

In this group both of its British representatives were secured for study, one of them, *Salix Andersoniana*, from several sources, both lowland and upland.

Salix Andersoniana, Sm. Of *Salix Andersoniana* material from several stations widely separated geographically was employed, and in every case the state of affairs was much the same as observed in *Salix aurita*—that is, we had seemingly perfect heterotype divisions with subsequent irregularities. Owing to the crowding of the chromosomes on the plates, the operations of counting and of size comparisons were alike rendered difficult. Nevertheless, our material uniformly gave the reduced chromosome number as fifty-seven. As for the individual chromosomes, nine could be recognized as definitely larger than the remainder (see Fig. 9, *a* and *b*).

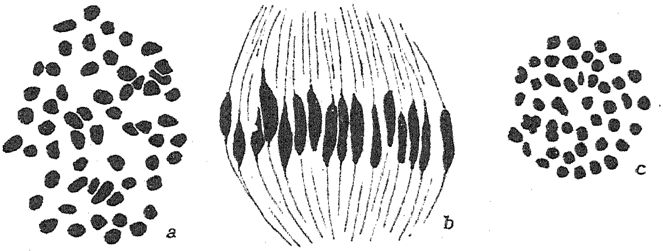

FIG. 9. Heterotype division. *a* and *b*. *S. Andersoniana*. *c*. *S. phylicifolia*.

In the homotype division, when disturbance was at its minimum, the number could similarly be made out. Often enough, however, at this stage, as in *S. aurita*, chromosomes were lost, failed to reach the plate, or behaved otherwise in an unusual manner, so that in many cases pollen abortion occurred, strongly suggesting the condition of a hybrid.

In somatic prophases, so closely packed were the chromosomes that the only fact determinable was that the number present exceeded one hundred.

Regarding nineteen as the fundamental number for the Order, *Salix Andersoniana* must be considered a hexaploid form.

Salix phylicifolia, Linn. Quite unexpectedly, although perfectly normal in other respects, *S. phylicifolia* gave in polar views of perfect heterotype plates a reduced number of forty-four, with which figure somatic counts of eighty-five–ninety were in complete harmony (Fig. 9, *c*).

This curious chromosome number brings the species into line with the case of *S. triandra*, and the explanation advanced to account for the situation there suffices in this instance likewise. If twenty-two can arise from a fragmentation of one compound chromosome in a diploid species, should a similar circumstance affecting two chromosomes take place in a tetraploid, a chromosome number of forty-four should be generated. Granting the truth of this view, then the second form of *S. triandra* emerges as a modified diploid species, whilst *S. phylicifolia* must be regarded as a modified tetraploid.

In any case, whether we regard the nineteen or the twenty-two as the original base number for the Salicaceae, we have a very interesting and suggestive occurrence indicating how it is possible for a second orthoploid series to be developed within the limits of the same Natural Order.

THE PHEN-HYBRIDS.

Of recognized *Salix* hybrids the males of three were prepared for study:

1. *Salix viminalis* × *S. purpurea* (*Salix rubra*, Sm.).
2. *Salix Caprea* × *S. lanata*.
3. *Salix aurita* × *S. phylicifolia*.

The first two crosses, judged by any deviation they exhibited from the customary series of events observed during meiosis in a phanerogam, might well have been pure species. In both cases the homology between the maternal and paternal chromosomes, as tested by their regular pairing in preparation for the reduction division and by the perfect nature of that division, was absolute, for not the least disturbance occurred to hint at hybridity. And this held true even to the appearance of the ripe pollen grains, which were massed, without the faintest signs of shrivelling or abortion, within the loculi. *Salix rubra*, resulting from a cross between two parents each endowed with a reduced chromosome number of 19, ought under the circumstance to possess a haploid number of 19, and such was the number displayed with unfailing regularity in good diakinetic figures, in perfect heterotype plates, and at the homotype divisions (see Fig. 10, *a*). Of similar import in this hybrid were the many somatic determinations, made in preparations from widely separated areas, yielding 38 as the somatic count.

Clearly, too, in the second hybrid, since *S. Caprea* has a haploid number of 19, and since the hybrid itself manifests the same reduced number, the other parent, *Salix lanata*, must likewise be of the diploid type (see Fig. 10, *c*).

Vastly different was the state of affairs in the third hybrid. There the parents were a tetraploid species, *Salix aurita*, on the one hand, and a modified tetraploid form, *S. phylicifolia*, on the other, so that theory demands the presence of 38 + 44 chromosomes in its somatic cells. This, in turn, entails that in the heterotype division, even if the chromosomes did assemble regularly on the equatorial plate, at least six should remain unpaired. As a matter of fact, about 32 bivalents as a maximum are to be found there, although the actual number varies between 21 and 32 (Fig. 11, *a*). To add to the confusion, the elements of the bivalents separate long before the laggard univalents approach the plate (see Fig. 11, *b*); consequently some whole or split univalents fail to be included in the daughter nuclei, which are thus constituted of widely different numbers of chromosomes, always short of the theoretical maximum. The aberrant individuals are usually included in micronuclei (see Fig. 11, *c*).

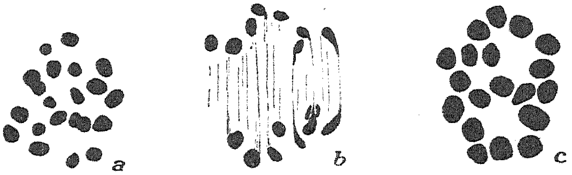

FIG. 10. Heterotype division. *a* and *b*. *S. rubra*. *c*. *S. caprea* × *lanata*.

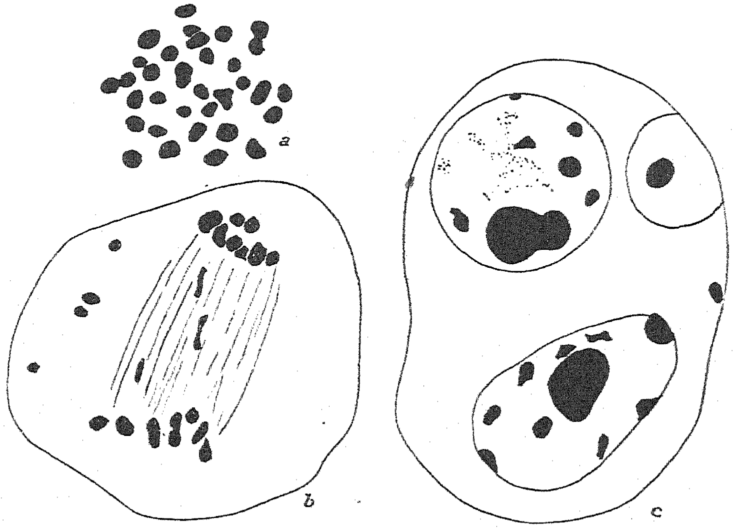

FIG. 11. *a–c*. Meiotic phase in *S. aurita* × *phylicifolia*.

Thus the more orderly phenomena observable during the pseudo-reduction in the Rosae are reproduced in a very incomplete sort of way, and then only in the closing chain of events. Comparisons, to be closer, should rather be made with the maturation divisions seen in the spermatogenesis of our *Biston* and *Oporabia* hybrids in the Lepidoptera.

In the preparation for the homotypical division once more matters pursue an unusual course; very rarely is there a perfect group of chromosomes on the equatorial plate, so that counts are made with extreme difficulty. With the generation of the four major nuclei the tetrad arises comprising four spores each with a major nucleus and one or more micronuclei. In general, however, contrary to one's expectations, the tetrad displays no great defects, and its members never exceed four in number. It is only later that degeneration resulting in a mass of almost impotent pollen ensues. Still, despite the parentage, and the almost wholesale abortion of the pollen, enough remains functional to allow for the development of more complex hybrids via this form.

CONCLUDING REMARKS.

One of the most remarkable facts in the foregoing work is the discovery that the fundamental number in the Salicaceae is 19. Except for isolated records provided by Ishikawa for a species of *Senecio* and by Maneval for *Liriodendron tulipifera* and *Magnolia virginica*, this number has never previously been detected amongst plants, and even in the former it appeared suspiciously in an orthoploid series based on five. Here, on the contrary, occurring in two genera and many species, it forms unmistakably the number upon which the series proper to the group depends. However, its importance is not confined solely to the novelty of the figure itself, but extends to the very significant departure from the systems, so uniformly encountered in plants, based on powers of two, three, four, five, seven, and combinations thereof. Nevertheless, the disagreement may be more apparent than real, for it is exceedingly probable that the chromosomes so often referred to as four-lobed, are actually compound. If such be the case, then reducing these to their constituent units and reckoning these with the genuine units, we may quite readily build up numbers like 24, 27, 32, 36, &c., all of which are of frequent occurrence in the phanerogams. That such a view is not far-fetched is proved by the presence of the second form of *Salix triandra*, in which one chromosome, apparently compound, has been broken into four conceivably of unit value.

Be that as it may, the development of the orthoploid series upon this base demonstrates that, whatever was true in the early history of the Order, the complexes, possibly modified in some species, are now absolutely stable. Thus we are brought to the fact that we have such a series with diploid, tetraploid, and hexaploid members as shown in the appended table:

		Salix.				
Populus.		*Pleiandrae.*		*Diandrae.*		
Diploid.	Tetraploid.	Diploid.	Tetraploid.	Diploid.	Tetraploid.	Hexaploid.
Populus tremulata, L.	*P. balsamifera*, L.	*Salix triandra*, L.	*S. lucida*, Mühl.	*S. daphnoides*, L.	*S. aurita*, L.	*S. Andersoniana*, Sm.
P. nigra, L.			*S. pentandra*, L.	*S. purpurea*, L.	*S. cinerea*, L.	
			S. pentandra var. *angustifolia*	*S. viminalis*, L.		
			S. fragilis, L.	*S. Caprea*, L.		
			S. fragalis var. *Basfordiana*	*S. repens*, L.		
			S. alba, L.	*S. myrsinites*, L.		
				S. lanata, L.		

In addition to this, and of equal value as demonstrating how a second orthoploid series may arise in one and the same genus, stands out the observation that *Salix phylicifolia* has a complement of 44 chromosomes, making it, on the basis of the lower member of its series, *Salix triandra*, a tetraploid form, or, on the base of 19, a modified tetraploid.

Now, if we are to judge from the result of hybridization experiments carried out by Wichura, Heribert-Nilsson, Ikeno, Linton, and ourselves, or from the hybrid combinations occurring naturally, the Pleiandrae form a group including species closely connected in their affinities and quite sharply isolated from the Diandrae, which, in turn, appear as a more or less homogeneous assemblage united by the tie of physiological similarity. This being so, we recognize at once that tetraploidy must have originated independently in the two supersections—a fact providing a very weighty argument in favour of the importance of the tetraploid species in evolution. Here we are confronted with the inevitable question: granting all this, how does the tetraploid species itself arise?

The first answers to suggest themselves seem to be those involving a duplication of the whole chromosome complement brought about by the inclusion of two newly-formed nuclei in one cell, or the transverse, longitudinal, or other form of fragmentation of the chromosomes without subsequent cell-division such as were postulated by Winge and others.

Favouring explanations of this type stand out the facts that Heribert-Nilsson, in propounding a satisfactory factorial analysis of his *Salix* crosses, had to assume the presence of duplicate factors, whilst we ourselves have had to make the same assumption with regard to the sex-determiners in seeking to explain anomalous sex-ratios.

Against these views can be brought the logical and at first sight unanswerable criticism that, even if all this were true, the circumstances are not such as to constitute a new species. At the most, it could be advanced, we have here a simple doubling of the genes borne by the chromosomes, and at the least, the same furniture of genes as the original plant, although now displaying a new grouping. Such an argument, however, contains a fallacy, for with new chromosome arrangement or furniture mere mechanical difficulties may occur to prevent, in some cases, free crossing with the parent type. Isolated in this manner, specific divergence of the tetraploid seems quite feasible.

Still, if the origin of the tetraploid lies in the methods just outlined, it must also by some means allow of the development and continued existence of the hexaploid species. Arguing from the analogy of *Primula kewensis*, we can perceive in hybridity a spur to such duplication of chromosomes (Digby). The new combination of characters once established, a second crossing with a third diploid species, succeeded by similar happenings, affords a mechanism for the evolution of the hexaploid. But, let it be noted, if hybridity is one of the causes of evolution, it cannot be the only one, for whence came the three original species the scheme demands?

At this stage we are enabled to answer the first question we proposed to consider. No matter whether diploid, tetraploid, or hexaploid, the behaviour of the *Salices*, both in micro- and megaspore formation, is very far from being that of F_1 hybrids, such as the majority of the Rosae seem to be. Only occasionally, as in *Salix fragilis*, *S. aurita*, and *S. Andersoniana*, are signs of hybridity given, and these, instead of indicating that they are cases of persistent F_1 hybrids reproduced apogamously like the Rose microgenes, probably represent traces of very recent crossing. In fact, observations in the field, directed from many angles, suggest that *Salix Andersoniana* is an F_1 or F_2 hybrid between *Salix phylicifolia* and some member of the Capreae group.

And this, coupled with the great affinity shown between maternal and paternal chromosomes in *Salix* crosses, affords a clue to the variability of the Salices, i.e. it results from the ability to cross, perfect within the limits of the two major groups, although somewhat lessened in its scope by the gregarious habits of most of the species, followed by an abundance of novel forms in F_2 and subsequent generations. This likewise enables us to explain why the characters in Heribert-Nilsson's *Salix* hybrids mendelized so perfectly. For, if the chromosomes provide the material and mechanical basis of heredity, judging from the cytology of the *purpurea-viminalis* and *Caprea-lanata* crosses investigated by us, the opportunities of segregation are not less than in the pure species.

With regard to our third query we have indicated above our belief in the existence of heterochromosomes in *Populus*. However, this investigation, in view of the negative results of other workers with dioecious plants, demands further attention, and this we are prepared to give.

Lastly, the irregular incidence of diploidy, tetraploidy, and hexaploidy in the various sections prevents those features in themselves from having any wide application in specific differentiation. On the contrary, quite unexpectedly, in the most critical cases, differences in chromosome number have been of extreme value. For instance, *Salix Caprea*, *S. cinerea*, and *S. aurita*, especially the first two, have been widely confused; yet the first is diploid and the second a tetraploid form. Similarly, very many Salicologists, and even Buchanan White amongst recent workers, have looked upon *Salix Andersoniana* and *S. phylicifolia* as specifically identical; our labours show that *Salix Andersoniana* is a normal hexaploid plant, whereas *S. phylicifolia* possesses the rather anomalous chromosome number of 44.

SUMMARY.

1. The fundamental chromosome number in the Salicaceae is 19.

2. Amongst the Salices examined were diploid, tetraploid, and hexaploid forms.

3. *Salix triandra* presented a form with 22 chromosomes as its reduced number as well as one with the normal number 19.

4. Falling in line with this second form of *Salix triandra* was *S. phylicifolia* with a reduced chromosome number of 44; thus a second orthoploid series is set up for *Salix*.

5. *Populus*, as far as our work goes, possesses diploid and tetraploid forms.

6. Only in *Salix fragilis*, *S. aurita*, and *S. Andersoniana* were abnormalities in meiosis discovered, and even then the departures from type were not great; nevertheless, they were such as to suggest hybridity.

7. In no case did these abnormalities recall those characterizing the Rosae.

8. Of the three recognized *Salix* hybrids examined two behaved quite normally during meiosis, whilst one did not.

9. The former observation explains why segregation occurred in Heribert-Nilsson's and Ikeno's *Salix* hybrids.

10. Hybridity, therefore, with segregation taking place in the F_1 and subsequent generations explains the variability of certain *Salix* species.

11. There is some evidence of the existence of heterochromosomes in the male of both *Populus* and of *Salix*.

12. *Salix Caprea* is easily differentiated from *S. cinera* and *S. aurita*, and *Salix Andersoniana* from *S. phylicifolia*, on the basis of chromosome number.

BOTANICAL DEPARTMENT,
ARMSTRONG COLLEGE,
NEWCASTLE-ON-TYNE.
August, 1923.

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On a Curious Effect of Mosaic Disease upon the Cells of the Potato Leaf.

BY

KENNETH M. SMITH, M.Sc.,

The University, Manchester.

With four Figures in the Text.

INTRODUCTION.

OWING to the great interest taken at the present time by plant pathologists generally in the problem of the so-called 'Virus Diseases' of plants, and especially because of the search for, and even reputed discovery of, a causal organism of a protozoal nature, the following short note may be considered opportune. During a critical search of the tissue of mosaic-infected potato leaves, the writer noticed the almost invariable presence, in the leaf cells, of a number of peculiar amoeba-like bodies. It is the purport of this note to give an accurate description of these bodies.

MATERIAL AND METHODS.

Both leaf and stem tissue of varying age from mosaic potatoes, in each case with a healthy control, were examined. Sections were cut both transversely and longitudinally, in thicknesses varying from six to ten microns, and made to pass through the yellow areas of the leaves. Various fixing and staining reagents were employed. The best results were obtained by using Flemming's fixing solution (weak) and staining with Flemming's triple stain. Good results were also obtained with Heidenhain's iron-haematoxylin. The drawings were made with the camera lucida, using a one-twelfth oil-immersion objective and a No. 4 ocular. The photomicrograph was taken with a one-twelfth oil-immersion lens.

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DESCRIPTION OF BODIES.

Scattered apparently indiscriminately throughout the tissue in the yellow chlorotic areas of the diseased leaf there were to be seen numbers of bodies varying somewhat in size and shape, but tending towards pear-shaped or round (Fig. 1). These corpuscles seemed to be possessed of a definite wall, and had in addition one or more very clearly defined vacuoles. They stained readily and presented a superficial resemblance to some kind of protozoal organism; in many cases they were in close association with the cell nucleus. Owing to this resemblance to a living organism attempts were made to cultivate these bodies, but without success, nor could they be shown to exhibit any movement or other sign of life. A careful examination of much material stained and fixed in various ways failed to reveal the existence of a nucleus in these bodies. Occasionally one or more small granules were discovered, but no definite statement can be made as to their nature. In the light green or yellow mottled areas to which these effects appear to be confined the general disintegration of the tissue seems considerable. The chloroplasts are much reduced in number, the cell-walls are ruptured, and the nuclei themselves are in a state of complete degeneration (see Fig. 2).

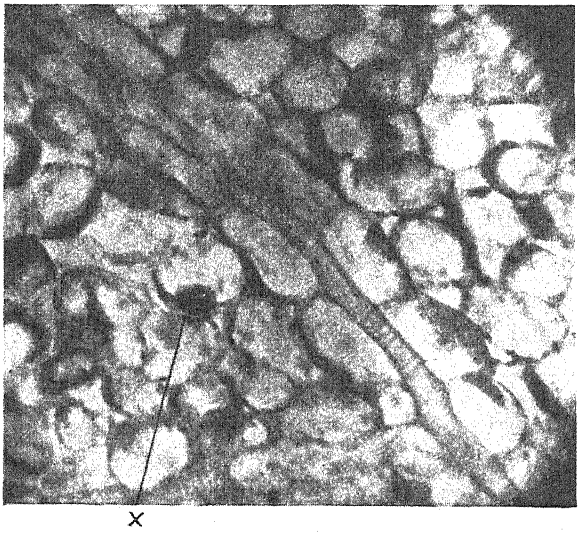

FIG. 1.

The explanation offered, then, of these curious bodies is that they are some kind of degeneration product of the cell, and most probably of the nucleus, induced by the mosaic, and that they are effects rather than causes of the disease. It seems likely that many of the 'organisms' and bodies described by various writers as associated with virus diseases of plants can be similarly explained.

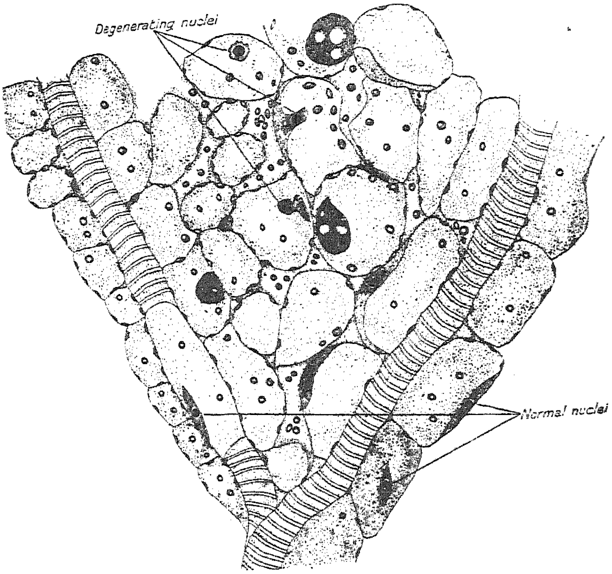

FIG. 2.

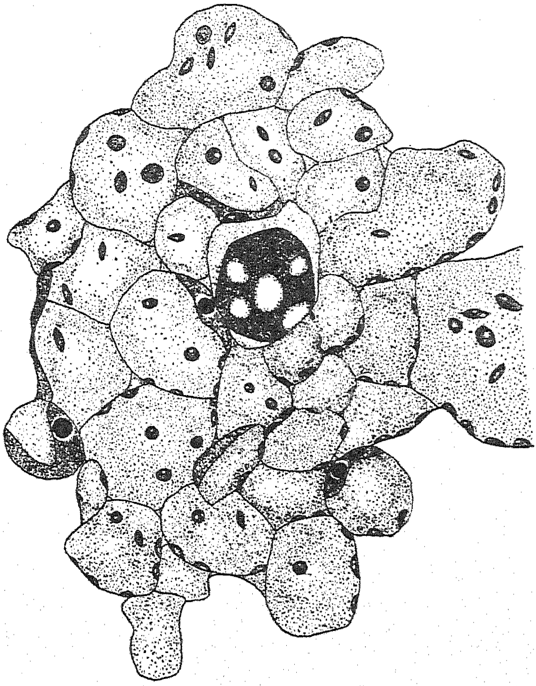

FIG. 3.

Fig. 1 is a photomicrograph of a section eight microns thick cut through the yellow area of a mosaic leaf of Golden Wonder potato. A pear-shaped body (×) with two vacuoles can be seen near the centre of the field.

Fig. 2 is a drawing of a section eight microns thick cut in the plane of the leaf surface, in the neighbourhood of a tracheide. It shows three bodies of varying types: one, pear-shaped, in the centre with two large vacuoles, and two small. To the left is a smaller body with one small vacuole, and a third is shown at the top of the drawing with three large vacuoles. Note the degenerating nuclei.

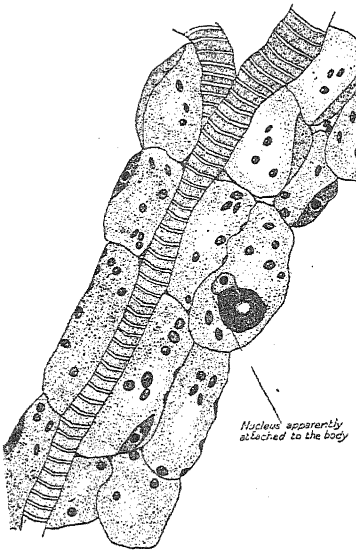

FIG. 4.

Fig. 3 is a drawing showing the presence of a single large body, with five vacuoles almost filling the cell.

Fig. 4 shows the close association of the body with the cell nucleus, which appears in this instance to be attached by a narrow neck.

NOTES.

THE PHYSIOLOGICAL ANATOMY OF MAYACA FLUVIATILIS.—The Mayacaceae are small perennial plants living in swamps and having the appearance of a moss such as of *Polytrichum*. About seven or eight species are known from North and South America and in south-western Africa.[1],[2]

This interesting, but small plant family has been very little investigated. From an anatomical point of view Poulsen[3] has studied the anatomy of the stem, leaf, and various organs of the flower in *Mayaca lagoensis*, Wing., and *M. Vandellii*, Scott et Endl.

As is the case in most submerged water-plants, the most striking feature of the anatomy is a well-developed aerenchyma. The species with which I worked differs considerably in various characteristics from those Poulsen studied, although other characteristics are much the same.

I carried out comparative physiological-anatomical observations on *Mayaca fluviatilis*, Aubl. (Fig. I, 1, 2, 3), which I collected in a swamp in the environs of Orlando, in the state of Florida, U.S.A. This species grows only in places where it has practically no competition with other species of plants, and but very few are to be found in its close proximity, among which I may mention two members of the Umbelliferae *Hydrocotyle americana*, L., and *Centella repanda*, (Pers.) Small. When, however, other species take a part in the association, *Mayaca fluviatilis* is usually unable to support the struggle for existence and disappears.

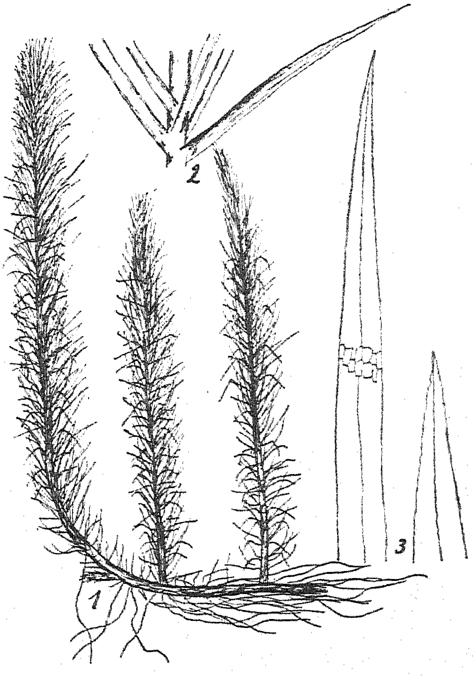

FIG. I. 1. Water form *Mayaca fluviatilis*, natural size. 2. Part of stem enlarged. 3. Leaves enlarged.

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[1] Engler, A., and Prantl: Die natürlichen Pflanzenfamilien. Berlin, 1889, Bd. 4.

[2] Uphof, J. C. Th.: Die Pflanzengattungen. Leipzig, 1910.

[3] Poulsen, V. A.: Anatomiska Studier over *Mayaca*. K. Dansk Vedensk. Selsk. Forhandl., Copenhagen, i, 1886.

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The numerous linear leaves are situated close to each other; they are placed spirally round the stem and apparently on the basis of the formula 5/13.

The whitish flowers are heterochlamydic and are borne on axillary pedicels. The ovary is one-celled, having three parietal placentae and a filiform style. The ovules are orthotropous. The capsule is three-celled, with three valves and placentae in the middle.

The plants are not confined to water, but with some care they can be raised as land plants as well, although such forms are rarely met with in nature.

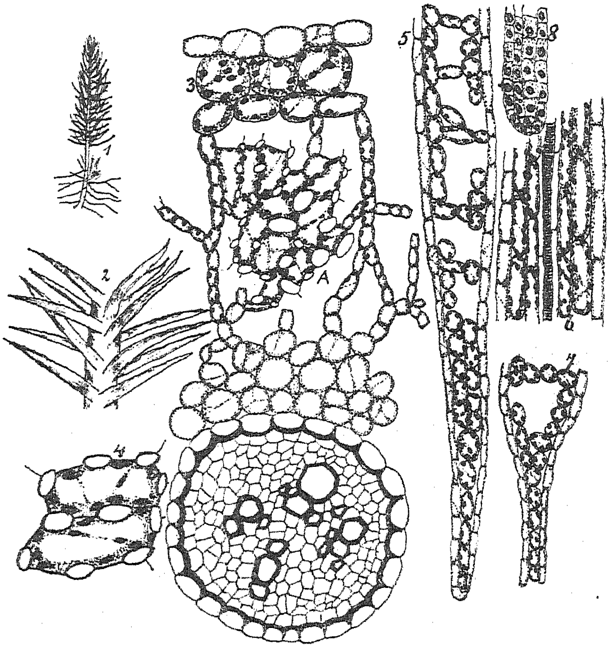

FIG. II. 1. Twig of land form. 2. A part of 1 four times enlarged. 3. Section of a stem; A, aerenchyma. 4. Star-celled tissue in the aerenchyma. 5. Longitudinal section of leaf. 6. Longitudinal section of vascular bundle in a leaf. 7. Section of a leaf of the land form. 8. Section of a very young leaf of the land form.

Investigations were directed to the behaviour of the various organs and tissues when surrounded by air and by water respectively. For this purpose a number of individuals were collected, and some grown in water and others in four-inch pots in which the soil was of the same composition, namely sandy mud, as that of their natural environment. In order to ascertain the exact behaviour of certain tissues in different environment, certain individual plants were divided in two, one part of which was carefully observed in water and the other part in a pot surrounded by air.

In neither case were there any difficulties in cultivation. The rate of growth during the first four months is shown in the following table:

Number of months.	*Growth in air.* mm.	*Growth in water.* mm.
1	8	20
2	12	40
3	18	72
4	25	90

In comparing the influence on growth of the two types of environment it can be easily observed that there is a great difference. The leaves grown in the air are considerably shorter as compared with those developed in the water. Also the thickness of the stem is less in air than in water; this becomes especially clear on measuring the tissues, as will be shown in the next table. Fig. I, 1 to 3, and Fig. II, 1 and 2, show the types of growth in both media.

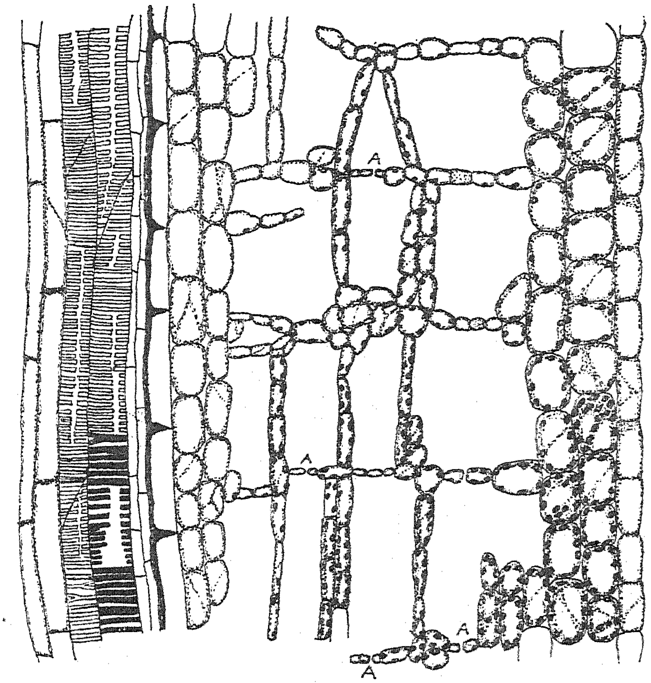

FIG. III. Longitudinal section of stem of the water form. A, aerenchyma.

The sections of the plant organs were made with a Spencer microtome and cut 15 μ in thickness. Considerable anatomical differences were to be observed in the different types of growth.

The stem (Fig. II, 3, and Fig. III) is surrounded by epidermis of large cells which are rich in protoplasm; this tissue is succeeded by a cortex having a thickness usually of three layers of cells possessing large characteristic chloroplasts. The next layer met with is a well-developed aerenchyma, which has been developed from the periblem. When the stem is very young the development of this aerenchyma can easily be traced from the latter tissue, in which certain cells divide only in the direction of the long axis and others only in the direction of the short axis of the stem; this consequently results in the formation of large and rather regular intercellular air-spaces.

Observing the aerenchyma closely it is evident that, besides the ordinary cell-tissue, one sees at various distances between the aerenchyma a kind of inter-tissue which is composed of somewhat star-shaped cells, which are relatively much smaller in size, as is shown in Fig. II, 3 and 4, and Fig. III. Through the small air-spaces in the small-celled tissue which occurs in the aerenchyma it is possible that the large intercellular air-spaces throughout the stem are connected one with the other.

The aerenchyma in the air form is developed only to two-thirds the extent of that in the watery environment, although there is apparently no other difference between the two aerenchymas.

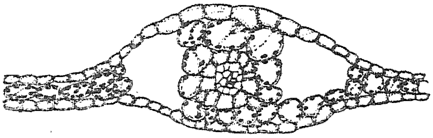

FIG. IV. Section of a leaf of the water form.

The aerenchyma is succeeded by three or four layers of round cells containing chloroplasts, which are also more strongly developed in the water form.

The endodermis is clearly visible and strongly developed, but there is an important difference in structure between the land and water forms. The thickness in the land form amounts to 10 to 12 μ, and in the air form 18 to 20 μ. Further, it is very obvious that the inner side of the endodermis is thicker in the land forms than in the water form; possibly the higher pressure in the fibro-vascular bundles requires a stronger developed inner endodermis wall.

There is apparently no difference in the form and size of the pericycle of the two forms; the cells are smaller than those of the endodermis; they are irregular. The same relationship can be observed in the elements of the well-developed phloem parenchyma and of the sieve-tubes. On the other hand, however, the protoxylem and the metaxylem offer sufficient differences in length and in width. The vessels are broader in the water plants, and consequently the entire vascular bundle has a greater diameter.

For comparison the measurements of various tissues are presented in the following table:

Thickness of Tissues or of Cells in the Stem.

Kind of Tissue.	*Air Plants.*	*Water Plants.*
	μ.	μ.
Epidermis	40	40
Cortex	60	80
Aerenchyma	400	600
Tissue round vascular bundle	50	100
Section of vascular bundle	130	200
a. Endodermis	10–12	20
b. Pericycle	5–9	4–10
c. Largest xylem vessel	17	25
d. Largest sieve-tube	4–5	4–5

The leaf (Fig. V) shows in a cross-section a small-celled epidermis on both sides, between which is found a mesophyll which has a thickness of but one layer of cells with but small intercellular air-spaces; towards the middle these cells become larger. In the centre is found a small vascular bundle (Figs. II, 6, IV, and V, 8), which is surrounded by large cells containing chloroplasts. Two large air-channels run throughout the length of the leaf (Fig. IV), which, like the fibro-vascular bundle, is smaller in land forms than in those developed in water.

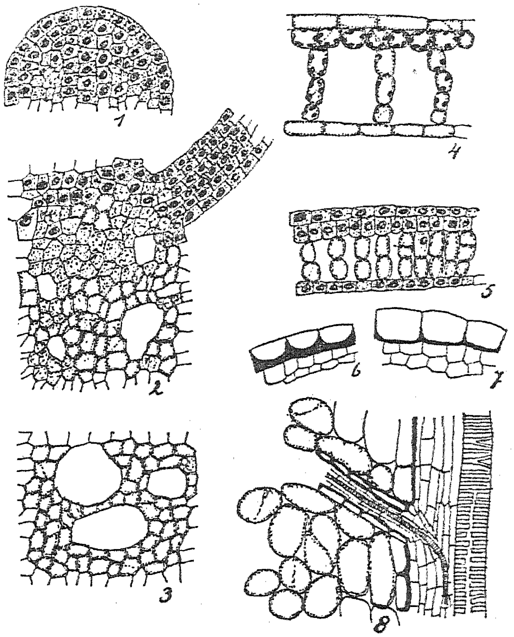

FIG. V. 1. Meristem of stem of the water form. 2 and 3. Same somewhat older, showing formation of aerenchyma. 4. Section of leaf of water form. 5. Very young leaf. 6. Endodermis of stem of land form. 7. Endodermis of water form. 8. Junction of two fibro-vascular bundles.

A section of a very young leaf (Fig. II, 8, and V, 2) shows one to sometimes five layers of cells which lie close together and have practically the same size. Some cells following the epidermis start dividing longitudinally, whereas some below these cells divide over their width, as can be observed in Fig. V, 4 and 5, causing the development of large air-spaces in the older leaf.

The construction of the root is very simple, and there is apparently no difference between the two forms. The epidermis encloses a cortex composed of but a few cells; this is succeeded by a triarch fibro-vascular bundle.

J. C. TH. UPHOF.

THE SWARMING OF ZOOSPORES OF PHYTOPHTHORA FABERI.—During the course of an investigation which necessitated the measurement of a large number of sporangia of *Phytophthora faberi*, it was noticed that swarming of zoospores rarely occurred. It is well known that the formation and emission of zoospores of the different species of *Phytophthora* are largely influenced by environmental conditions. Usually, the discharge of zoospores begins about fifteen minutes after the sporangia are placed in water at a suitable temperature. Coleman[1] considered a certain strength of light to be the chief factor influencing the formation and emission of zoospores of many Phycomycetes; he found that the sporangia of *P. arecae* swarmed freely when suspended in water and placed on the stage of a microscope and illuminated by a mirror and condenser. *Phytophthora faberi* under these conditions did not produce zoospores at all readily, and when swarming did occur, it was usually from sporangia situated near the edge of the cover-glass or in close proximity to an air-bubble. In tube cultures the emission of zoospores into water which had condensed on the sides of the tube was common, and large numbers of empty sporangia were to be found in all cultures.

The following experiments were carried out to determine the conditions favouring the discharge of zoospores of *P. faberi*. The sporangia for these experiments were obtained from pure cultures grown on cacao pods in the laboratory.

I. *Time.* A suspension of mature sporangia in fresh tap-water at 22° C. (room temperature) was placed in a flask near the window. Swarming of zoospores commenced after fifteen minutes. After two and a half hours swarming had ceased, and no active zoospores were to be found in the water.

Corrosive sublimate solution was added to a small sample of this suspension to prevent further swarming. Other samples were taken at quarter-hour intervals and treated similarly, until swarming had ceased in the flask—two and a half hours after the commencement of the experiment.

The samples were later examined microscopically to determine the proportion of sporangia which had discharged their contents. Between 500 and 600 sporangia were examined from each sample, and the following percentages of empty sporangia obtained from the different samples:

	30	45	60	75	90	105	120	150 min.
Percentage empty	8·4	9·2	17·1	26·4	28·9	33·2	39·3	39·6

Less than 40 per cent. of the sporangia discharged zoospores within two and a half hours, and of these the majority had swarmed within one and a half hours.

II. *Temperature.* The effect of temperature on the swarming of *Phytophthora* zoospores has been noted by various writers. Dastur[2] observed that zoospores were emitted from sporangia of *P. parasitica* in five minutes at 25° C., and that higher temperatures retarded the zoospore formation. Melhus[3] found temperatures below 20° C. more favourable for zoospore formation in *P. infestans* than higher temperatures.

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[1] Coleman, L. C.: Diseases of the Areca Palm. Annales Mycologici, viii. 591–626 (1910).

[2] Dastur, J. F.: On *Phytophthora parasitica*. Mem. Dept. Agric. India, v. 177–231 (1913).

[3] Melhus, I. E.: Germination and Infection with the Fungus of the Late Blight of Potato (*Phytophthora infestans*). Wisc. Agr. Exp. Sta. Res. Bull. xxxvii. 64 (1915).

Suspensions of spores were made in tap-water at 5, 10, 15, 20, 25, 30, and 35 degrees centigrade, and these temperatures maintained for $2\frac{1}{2}$ hours. At the end of that time the suspensions were killed with corrosive sublimate and examined as in the previous experiment, 500 spores being taken as a sample. As the culture from which the sporangia were taken was rather old, a determination of the number of empty sporangia in the suspensions at the commencement of the experiment was made and found to be 4·2 per cent. The percentages of empty sporangia found in the suspensions after $2\frac{1}{2}$ hours are given below and represented graphically in Fig. 1:

	10°	15°	20°	25°	30°	35° C.
Percentage empty	4·4	5·8	19·8	18·6	4·2	3·8

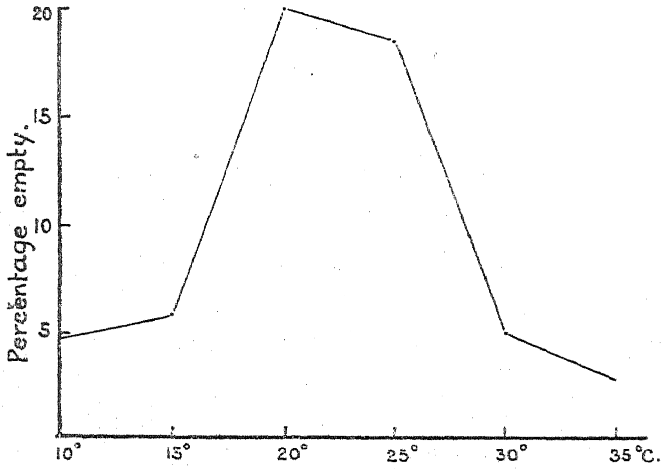

FIG. 1. The effect of temperature on zoospore formation in *P. faberi*.

The optimum temperature for zoospore formation in *P. faberi* therefore lies between 20 and 25° C. At temperatures below 20° C. or above 25° C. the formation of zoospores is retarded.

III. *Light and Air*. A suspension of sporangia in tap-water was divided into two parts. One was placed near the window and the other in a dark chamber. Samples were taken from the flask near the window at quarter-hour intervals after the first half-hour and killed with corrosive sublimate. From the flask in the dark, samples were taken at half-hour intervals in order to avoid admitting light too frequently.

A suspension of sporangia from the same culture was made in tap-water which had previously been boiled and allowed to cool. The suspension was divided into five parts and placed in small flasks near the window. Corrosive sublimate was added to a flask every half-hour; each flask therefore constituted a sample. Care was taken throughout to avoid aerating the suspensions.

The temperature of these suspensions varied from 22° C. at the beginning of the experiment to 23·5° at the conclusion.

Later, 250 sporangia were examined from each sample to determine the proportion of empty sporangia; the results are given in the following table:

Percentage of sporangia which had discharged zoospores at various times in suspensions (1) *in the light*, (2) *in dark*, (3) *in the absence of air.*

	30	45	60	75	90	105	120	135	150 min.
In light	10·8	34·4	50·0	56·4	60·4	62·4	67·8	66·6	66·0
In dark	10·8	—	18·4	—	33·8	—	38·4	—	41·6
Without air	6·8	—	6·4	—	15·8	—	10·2	—	10·4

These results are represented graphically in Fig. 2. It is evident that, in the absence of dissolved air in the water, very little swarming of zoospores occurs.

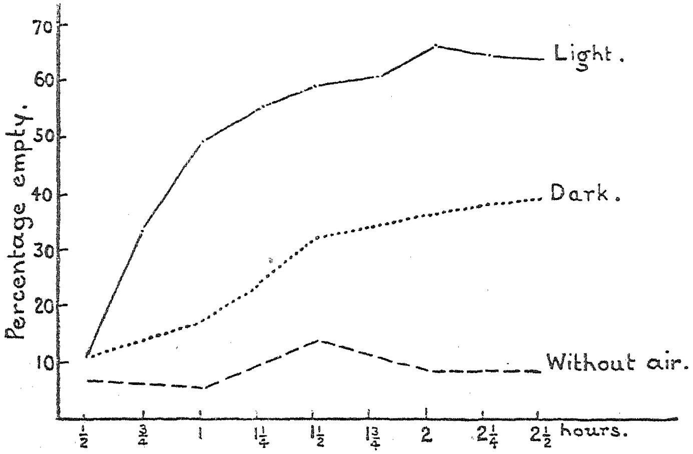

FIG. 2. The effects of light and air on the emission of zoospores of *P. faberi.*

The necessity of a requisite supply of air for the formation and emission of zoospores has not generally been recognized. Klebahn's[1] observations on the swarming of zoospores of *Phytophthora Syringae* are of interest in this connexion. Sporangia were first observed after growing the fungus in pure water. These sporangia, however, did not discharge zoospores, and it was only after the water of the culture was replaced by fresh water saturated with oxygen that Klebahn succeeded in inducing zoospore formation; then swarming began after a quarter of an hour. The difficulty experienced by various investigators in obtaining swarm-spores at will is possibly connected with the oxygen requirements of the species.

The culture from which the sporangia were taken for this experiment had just commenced fruiting, and was younger than those used for the previous experiments. This may account for the greater percentage of sporangia which discharged zoospores in the presence of light than occurred in the previous experiments.

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[1] Klebahn, H.: Krankheiten des Flieders. Berlin (1909).

Though a smaller percentage of sporangia emitted zoospores in the absence of light, these results cannot be considered conclusive. Before pouring samples, the flasks were shaken to obtain even suspensions. As more examples were taken from the suspension in the light, it was consequently more frequently shaken and the water better aerated than that in the dark. This of itself might account for the different proportions of empty sporangia in these suspensions. A further experiment was therefore carried out to determine the influence of light.

IV. *Light.* A suspension of sporangia was made in tap-water at 22° C. This was poured into two small flasks, one of which was placed near the window and the other well covered with black paper to exclude the light. At intervals the flasks were well shaken to allow thorough aeration. After two hours further swarming was prevented by the addition of corrosive sublimate. One thousand sporangia were examined from each flask; 297 had discharged zoospores in the light, and 226 in the dark, showing a difference of seventy-one in favour of light. Since this difference is 3·6 times its standard error (19·6), it is very unlikely that it had arisen as a mere error of sampling; it must therefore be considered as significant.

Dastur (loc. cit.) found that light was not only essential for the formation and emission of zoospores of *P. parasitica*, but also for the formation of sporangia. *P. faberi* does not appear to be so closely dependent on the presence of light as is *P. parasitica*, as cultures of *P. faberi* produce sporangia in the absence of light, though not so abundantly as when light is present.

Conclusion. The conditions favouring the formation and emission of zoospores of *P. faberi* are—(1) suitable temperature, (2) well-aerated water, and (3) light. The presence of light, however, does not appear to be as important a factor with *P. faberi* as has been shown to be the case for *P. arecae* or *P. parasitica*. The age of the sporangia would also appear to be important, the highest percentage of swarmed sporangia being obtained from young cultures.

C. H. GADD.

PERADENIYA, CEYLON,
September, 1923.

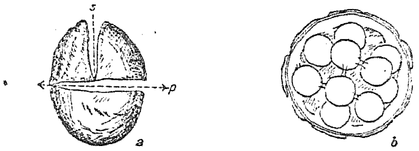
SOME FEATURES OF THE ENVELOPE IN COELASTRUM.—The colonial Green Algae, which reproduce by means of autocolonies, include a series of genera showing some of the simplest possible types of colony organization, and are consequently closely related to the purely unicellular genera which reproduce by means of autospores. The genus *Coelastrum* is characterized by having the cells of the colony arranged in a simple hollow sphere or polyhedron. It has also been obtained in the unicellular form (1, 3, 5). The character of colony formation and its origin, therefore, appear to be of a very simple nature in this genus.

The adhesion of the cells resulting in the colonial character depends on the structure of the individual cell envelopes. In *Coelastrum*, as in *Scenedesmus*, an inner layer staining with cellulose reagents can be distinguished from an outer pectic layer staining with aqueous fuchsin and tannic acid vesuvin. Senn (5) found that the parent cell membrane, on liberating the offspring cells, splits into two hemispheres only attached at the point nearest the centre of the original colony. They finally dissolve, and do not take part in the formation of the offspring colony as had been assumed by earlier workers. Soon after division the two layers of the wall can be distinguished in the offspring cells, which, according to Senn, produce them anew in each generation. The offspring colonies may remain attached, however, to the basket formed by the cell-walls of the previous generation; sometimes three generations may thus form a single compound colony, but in most cases the offspring have dissociated before this stage.

Further observations on the envelope were made by Rayss (3). The production of isolated cells was studied under experimental conditions by this author. Since in the colony the cells are attached together by means of mucilage belonging to the outer layer of the cell-wall, the process of dissociation, whether congenital or postgenital, must be regarded as a variation of the envelope. Rayss also figures simple variations in thickness of the outer layer and the occurrence of stratification in some cases. Senn had previously noted that the cellulose layer was thicker and sometimes two-layered in hypnocysts. In some of the most remarkable variants, however, the outer layer is not uniformly distributed over the surface of the cell, but appears in the form of separate discs. Rayss found such variants in cultures containing glucose, and compares them with similar forms observed previously by Chodat (1) in *Pediastrum* in a concentrated saline solution, and in *Protococcus* and other Algae.

The different species of *Coelastrum* are distinguished by differences in the character of the envelope and the form of the cells. The former are of interest here in relation to the phenomena to be described below. In *C. microporum*, Naeg., the cell-wall is practically of uniform thickness. In others the outer layer is produced into equatorial processes, connecting the cells together, or into polar outgrowths. The former reach their maximum development in *C. reticulatum*, (Dangeard) Senn (*Hariotina*, Dangeard). In the genus *Scenedesmus* connecting processes occur, although the majority of species do not possess them. They are especially well developed in *S. tropicus*, Crow, where they are clearly of the same morphological nature as in *Coelastrum*. Polar thickening occurs in *C. cambricum*, Archer, and is well marked in *C. proboscideum*, Bohlin. The large mucilage outgrowths of the related *Burkillia cornuta*, W. and G. S. West, are a further expression of this tendency. It is noteworthy that *Scenedesmus* presents analogous phenomena. The cells of many species of the latter genus are provided with spines and teeth which are mere modifications of the outer layer of the envelope.

In *C. Morus*, W. and G. S. West, the thickenings of the cell-wall take the form of papillae. *C. schizodermaticum*, Rich, shows this type of envelope with the additional feature of exfoliation of portions of the membrane. The papillae appear to correspond with the connecting processes of other species, although here the cells cohere by broad flattened surfaces. Small circular or four-cornered caps are detached by fission of the outer layer of the cell-wall along lines connecting the papillae (4). Rich suggests that the cell-wall may become more mucilaginous at the points where the pads are formed, and this may give rise to the splitting off of the caps.



a. Cell-wall after liberation of offspring colony. *p*, primary plane of fission; *s*, secondary plane of fission. *b*. Single cell of parent colony before liberation of offspring colony. The cell-wall is shown in optical section in *b*.

Whilst examining collections of freshwater plankton made by Professor F. E. Fritsch in Ceylon, and which have been described elsewhere (2), *C. reticulum*, (Dangeard) Senn, was recorded from the lake at Panadure. Some of the colonies were typical vegetative colonies of this species, other specimens were in reproduction by means of autocolonies. Further observations on the latter show what appears to be a previously unknown phase in the development of the cell-wall of *Coelastrum*. Amongst the specimens concerned traces of the connecting processes characteristic of *C. reticulatum* were found in the young colonies as in the figure, so that they may be assigned to this species. The parent cells had a diameter of 12 to 21μ, and the offspring cells measured 3 to 6μ. In colonies where the cells had already undergone division to produce offspring colonies, but in which the membrane had not yet split open to liberate the contents, an incomplete splitting was observed, as shown in the figure above. The outer layer had broken up into a number of small segments which could be particularly well seen on mounting in glycerine jelly. Some of the segments appeared to be becoming detached. The phenomenon is in fact similar to the segmentation and exfoliation of the membrane in *C. schizodermaticum*, but the latter is not associated with the process of division. The caps, too, were not so extensive as in *C. schizodermaticum*; in number they roughly equal the cells in the offspring colony. Their arrangement is a regular one, like that of the thickenings in *C. Morus*, although flatter than the latter structures. Apart from their regularity they might also be compared with the discoid thickenings in the forms figured by Rayss and referred to above. It is doubtful whether the segmentation observed has any functional connexion with the subsequent splitting of the membrane to liberate the offspring colonies, although structurally comparable with it. In one empty membrane, however, a secondary plane of fission (*s* in figure) was represented in one of the hemispheres separated by the primary plane of fission (*p* in figure) and approximately at right angles to the latter.

In some unicellular Isokontae the membrane of the sporangial cell separates into a number of portions corresponding with the number of spores formed. In others the membrane merely divides into two hemispheres, whilst four, eight, or more offspring cells are formed. The latter occurs frequently in species of *Coelastrum*. The variants described above are therefore of interest in showing the incipient development of the former type in the genus *Coelastrum*. In still other autosporous forms the conversion of the cell-wall to mucilage sets in concurrently with cell-division, so that splitting does not occur.

In the phase of development figured (*b*) the envelope of *Coelastrum* is comparable with that of *Schizochlamys*. In both the outer portion is segmented, but in *Schizochlamys* the inner layer is represented by a wide mucilage sheath, the persistence of which leads to the adhesion of the colonies into compound aggregations or thalli which may attain macroscopic dimensions. This difference must not be regarded as excluding the possibility of close relationship between *Coelastrum* and *Schizochlamys*, since the change of a membrane to a layer of mucilage is of very common occurrence in the autosporous Chlamydomonads. *Coelastrum* and *Scenedesmus* both exist in a palmelloid phase (1, 5), whilst in the related *Nephrocytium* a definite *Schizochlamys* state is described (1). The segmented portions in *Schizochlamys* have the microchemical properties of the outer layer in *Coelastrum*, and according to Chodat (1) do not show the reactions of cellulose. The mucilage layer of *Schizochlamys*, moreover, appears before cell-division of the contained protoplast, and does not therefore belong to the daughter-cells. Splitting generally takes place about the time of cell-division, but is not always correlated with it. *S. gelatinosa*, A. Br., for instance, often shows considerable splitting of the membrane in undivided cells. Frequently the number of segments corresponds with the number of cells, but this is by no means invariably the case. *S. hyalina*, Fritsch, is remarkable for the varying number of pieces.

The mode of development of the envelope in *Oocystis* shows certain comparable features. In this genus, as in *Coelastrum*, an internal cellulose layer and an external pectic layer are represented. In many species of *Oocystis* there are thickenings of the outer layer, generally two in number, one at each end of the ellipsoidal cell. When the cell divides the membranes are converted into mucilage, but the thickenings remain as prominent caps at the poles (1). A similar mechanism is evidently at work in *C. schizodermaticum*, Rich, and in the form figured here, the amount of mucilage being much less in these latter types. It is evident that in *Coelastrum* and allied genera the process of splitting is a secondary result of differentiation of the various layers. The strong tendency towards localized thickening which is shown in the comparison given above of the envelope structure in different species is another important factor. The very varied types of structure in the genus can thus be reduced to very simple terms, and therefore do not involve important systematic differences.

SUMMARY.

Summary. A new type of variation in the structure of the envelope of *Coelastrum* is described, and certain relationships between it and other known types of envelope structure are pointed out. A number of apparently diverse modifications in the envelope of *Coelastrum* and other genera are thus elucidated, and are found to agree in essential morphological features.

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W. B. CROW.

TWO MYCOLOGICAL METHODS. I. A SIMPLE METHOD OF FREEING FUNGAL CULTURES FROM BACTERIA.—When the inoculum from which a fungal culture is to be started is contaminated with bacteria, as is often the case with inocula derived from nature, it is desirable to make the sowing on a medium which will allow rapid surface growth of the fungal colony, while at the same time slowing down the growth of the bacteria. For this purpose acidified media are often used. The writer finds that a medium of plain agar (15 grains agar to the litre) has many advantages. The rate of surface growth of many fungi on plain agar is as fast as, and in many cases faster than, that on a richer medium, the effect of the feeble conditions of nutrition being shown merely in the thinness of the fungal mycelium. Under such starved conditions, however, the bacteria multiply only to a limited extent, and thus a concentration of bacterial excrete products sufficient to interfere with the growth of the fungus colony is not reached. The converse effect is seen in rich neutral media, where the bacteria multiply so rapidly that the fungal spores may even be prevented from germinating.

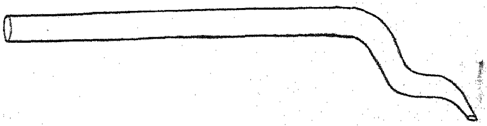
A fungus which is contaminated with bacteria will often, when sown on plain agar, grow away from the bacteria, so that all that is necessary in order to obtain a clean culture is to transfer from the growing edge of the colony. In other cases, however, this is not possible, as a thin film of bacteria surrounds the hyphae right up to the growing tips. Now it has been observed that the bacteria are confined to the surface of the medium, so that a transfer taken from below the surface is always bacterium free.

The method of procedure is therefore as follows: A Petri dish of plain agar, the layer of gel being about half a centimetre deep, is inoculated in the centre with the fungus in question. When the colony has grown for about five or six days the medium is cut through with a sterile knife in advance of the growing edge, and the colony is turned over. The inoculum is then taken from the original under surface, care being taken not to break through to the other side.

The present method would obviously fail in any case where the fungal hyphae do not grow down into the medium or where the bacteria do so. No such case has so far been met with by the writer, so that in all probability the method is of very general application.

Fungi such as *Saprolegnia*, *Pythium*, *Fusarium*, &c., which on isolation are generally contaminated with bacteria, are readily cleaned in the manner described.

II. A METHOD OF ISOLATING SINGLE STRAINS OF FUNGI BY CUTTING OUT A HYPHAL TIP.—The method is modelled on that of Edgerton[1] for picking up single spores. The main requirement is a microscope with a substage condenser which can be swung out. The condenser is removed, and in its place is inserted a cork with a deep groove cut in the upper surface. Into this groove is wedged vertically a spring clip (such as is used with a burette). A glass tube is fixed more or less horizontally in the clip, so that it is above the stage and reaches to the field of the microscope. In order to confer the necessary rigidity, two notched pieces of brass are soldered to the clip, and between these the glass tube passes. A metal rod is clamped in a vertical position to the edge of the stage and projects below it. The condenser carrier is held against this rod by means of a rubber band, so that when the condenser screw is turned the apparatus is constrained to move up and down at right angles to the stage. The glass tube is shaped as in the diagram above, the capillary end being cut or ground, so that when



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[1] Phytopathology, 1914, iv. 115–17. The mechanism described is similar to Edgerton's, and a reference to the illustration given by the latter will show the general appearance of the apparatus.

the tube is in position the capillary orifice is horizontal. The tube is so shaped to avoid fouling the objective of the microscope and also the sides of the Petri dish containing the fungal culture.

In cutting out a hyphal tip one proceeds as follows: The condenser holder is raised to its highest point and the capillary orifice centred in the field of the low-power objective. This is readily effected by sliding the tube through the clip and rotating it on its axis. A Petri dish culture of the fungus is placed on the stand of the microscope, and the capillary tube lowered till nearly in contact with the medium. The tube is then swung out of the field, and an examination made of the margin of the colony until a hyphal tip is found so sufficiently isolated that it can be picked out by the capillary. In this examination the deeper layers of the medium must also be carefully explored. The capillary is then swung back, and after it is seen that the orifice covers the particular hyphal tip and no others, the tube is plunged down through the medium by screwing down the condenser holder. A plug of medium carrying with it the hyphal tip is thus forced up into the capillary. The tube is then withdrawn and the plug blown out into a tube of sterile medium, where the presence of a single hyphal tip may be confirmed by microscopical examination through the glass.

The success of this method depends on growing the fungus in such a way that a single hyphal tip is readily isolated. The following is the technique of dealing with certain *Fusarium* strains. The details will probably require to be modified somewhat for other fungi.

The fungal inoculum—usually in the form of spores—is placed on a small plate of plain agar. With this medium the density of the hyphae is reduced to a minimum. The colony is allowed to grow to such a size that its margin is well away from the original inoculum, some of the spores of which may remain ungerminated. Even in such a thin colony it is in general impracticable to pick up readily a single hyphal tip. Using the apparatus as above described, a plug of medium is picked up from the edge containing only a few hyphal tips. This plug is then transferred to another part of the plate. After a lapse of about one day these few hyphal tips, having the whole circumference in which to spread out, are found to be so widely spaced that the picking out of a single tip is an easy matter.

The process has the advantage of being very expeditious, and with practice all the operations become mechanical. With cultures at the right stage it is possible to carry out the operation of picking up a single tip in a few minutes, so that a batch of 40 or 50 cultures can easily be carried through in a day. Between each operation the capillary tube is placed in a beaker of boiling water.

In view of the very small area of the medium picked up, the risk of contamination from air-borne spores is very small. However, as a precaution against contamination the writer always disinfects beforehand the room in which these operations are to be carried out. Out of several hundred hyphal tip cultures which have from time to time been prepared in the manner described, only in one case did contamination from an external source appear.

In dealing with a mixture of two fungi the method has the disadvantage that it only isolates the faster growing constituent. It is applied to greatest advantage in the setting up of cultures which can be guaranteed free of admixture with any other organism. For this purpose it is, at least in the case of *Fusarium*, much superior to any of the ordinary spore methods. The best of the latter methods are very laborious, even in the case of organisms with large spores. With organisms which possess very small spores the labour involved in obtaining a culture which can be guaranteed to arise from a single spore is often excessive, and when spores of different sizes which may belong to one or more than one organism, are present, the spore method is fraught with great danger, as a small spore, if slow to germinate, could easily be passed over when lying in the neighbourhood of a more quickly germinating large spore. In the case of sporeless fungi the hyphal tip method is, of course, the only one available. For the general purpose of obtaining pure cultures and for the particular purpose for which it was elaborated, viz. the separation of saltants in a saltating culture, the hyphal tip method is in the writer's experience much superior to any of the usual methods, both as regards ease of manipulation and certainty of result.

W. BROWN.

IMPERIAL COLLEGE OF SCIENCE
AND TECHNOLOGY, LONDON.

NOTE ON THE PREPARATION OF HERBARIUM MATERIAL FOR HISTOLOGICAL STUDY.—In the study of floral development it is often desirable to compare not only the adult materials with type specimens, but also to secure whatever information can be drawn from the earlier floral stages, which are frequently well preserved in herbarium specimens. It has been matter for some surprise that while adult flowers are so commonly damaged in herbarium specimens by desiccation and pressure, the most minute flower-buds, though compressed and brittle, may retain their chief histological features, and may be open to detailed study with results which compare not unfavourably with those obtained from recent materials fixed in alcohol alone. The method of treatment of herbarium materials under investigation here discussed is not in its essentials novel, but the results secured have been so satisfactory, even with flower-buds pressed towards the close of the eighteenth century, and from which a full developmental story has been secured, that the routine followed in our work seems worthy of record.

The materials are immersed in a mixture of 5 to 10 parts of sodium hypochlorite solution and 95 to 90 parts of water, and kept therein at room-temperature for from two to ten days until a complete penetration and softening of the tissues is indicated by their transparency. The danger of over-softening is so great when the penetration is hastened by the employment of a strong solution of sodium hypochlorite, and the advantage of accelerating the penetration by heat is so doubtful, that a slow action with weak solution has been relied upon and has fully justified itself.

It seems desirable to note, lest any confusion should arise, that sodium hypochlorite (NaOCl) is similar in constitution to eau de Labarraque, and may be purchased in the solution above referred to, which has approximately five times the strength of the latter. When the materials are fully bleached they are as a rule so soft that it is essential to avoid handling them in the washing which follows. A suitable receptacle, in which not only are the materials washed with safety in running water, but are later dehydrated, stained in bulk if desirable, and impregnated with xylol, is a glass tube some three inches in length, about one inch in diameter, and open at both ends. A piece of fine gauze is stretched and tied over both ends, ensuring free water circulation within the tube during washing and avoiding unnecessary handling of the materials contained in the tube while they are still soft.

It is found advantageous in the dehydration which follows washing to employ a series of 6-oz. glass-stoppered, wide-mouth bottles containing from 2½ per cent. to absolute alcohol as indicated in Chamberlain's Methods, 1915, p. 105. Glass beads placed in the bottoms of the alcohol-bottles aid in the sedimentation of debris which accumulates rapidly during dehydration. The tubes in which the materials have been washed are immersed in the succession of alcohols. The materials are as a rule sufficiently hardened before their entry into the series of xylols to ensure their safe handling in the later stages of preparation.

W. HORTON.

HARTLEY BOTANICAL LABORATORIES,
LIVERPOOL UNIVERSITY.

The Cytology of the Cotton Plant.

I. Microspore Formation in Sea Island Cotton.

BY

HUMPHREY JOHN DENHAM, M.A. (OXON.), F.R.M.S.

(*The British Cotton Industry Research Association*).

With Plates XI–XIV.

INTRODUCTION.

IN the present paper the cytology of pollen formation in Sea Island cotton (*Gossypium barbadense*, var. *maritima*, Watt) (1) is briefly described, with special reference to the reduction division of the pollen mother-cell and chromosome number. These observations form part of a series of investigations on the cytology of the various cottons in cultivation; their bearing on the problems of scientific cross-breeding and improvement of commercial cottons forms the subject of a separate paper in preparation, and therefore need not be discussed here.

Two papers only have been published on the cytology of *Gossypium*, neither of which deals with the type under consideration, though the forms discussed are sufficiently homologous to admit them for comparison. The first, published by Cannon (2) in 1903, deals with the cytology of a hybrid between commercial Sea Island and Upland plants, with special reference to the production of nuclear abnormalities by special crossing. The cytology of the parents was apparently not studied, but the general outline of the nuclear history agrees fairly consistently with the known behaviour of the sporocyte in other Angiosperms, and with the observations below. Some 19 figures are given, many of them of abnormal conditions, and the chromosome number of the hybrid is stated at 28 (haploid).

The second paper, by Balls (3) in 1910, which deals with the cytology of the Egyptian *Mit Afifi* cotton (a commercial type approximating to *G. barbadense*), is chiefly an attempt to establish a mechanism of nuclear

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division which would account for the absence of the centrosome in the higher plants, and describes certain 'thread-ring' structures which are supposed to unite the ends of the spindle fibres, persisting through the life of the nucleus, and taking an important part in the behaviour of the spireme; one plate is given, and the haploid chromosome number is stated as 20. This 'thread-ring' theory of karyokinesis seems to have escaped the notice of later writers, and the structures have not been described in any other plants. Details of the developmental history of the flower, and of the micro- and mega-sporangia may be found in an earlier paper by the same writer (4).

MATERIAL AND METHODS.

Some 200 plants were available in the Shirley Institute experimental greenhouse, raised from seeds of pedigreed pure strains which had been brought from St. Vincent by Dr. S. C. Harland. This material had in every case been self-fertilized for at least six generations, and was in consequence homozygous to a very high degree. A certain amount of material from other sources was also available for comparison, including Trinidad native types, commercial Uplands, and Egyptian *Mit Afifi*, though no haploid material of the latter was obtainable during the period of these investigations. A large number of fixing solutions were tested on buds and root-tips, and it was found that the most satisfactory preparations were given by von Tellyesniczky's potassium dichromate-acetic acid mixture; the addition of small quantities of osmium tetroxide gave slightly sharper chromosome definition, but could not be used in all cases on account of the presence of fats and lipoids. Fixation was carried out at the temperature of the greenhouse, the buds being first stripped of their involucres to facilitate rapid penetration of the fluid. No improvement was produced by fixing under reduced pressure. For dehydration, the recently published technique of Mlle Larbaud (5) was used, in which commercial butyl alcohol is substituted for the higher grade alcohols and xylene mixtures, with very marked improvement. Approximately correct diameters of buds for fixing are as follows (involucres removed):

Synapsis, 3 mm.; diakinesis, 3·5 mm.; tetrad spindles, 4 mm.

When a considerable number of buds had been cut, it was found that late anaphase stages were almost completely absent. It appears from the researches of Laughlin (6), that in *Allium* root-tips the anaphases are accomplished considerably more quickly than the other stages, and it would appear that in the present case chromosomes entering on anaphase pass into telophase before they are reached by the fixative. To overcome this, a modification of Carnoy's fluid was used (ethyl alcohol 40 per cent., glacial acetic acid 40 per cent., chloroform 20 per cent.) with complete success.

The material after fixation was taken through a graduated series of alcohol-chloroform mixtures into pure chloroform, to which paraffin wax was added gradually, and finally transferred to pure paraffin wax for embedding. This gives excellent nuclear fixation, though slight distortion of the cytoplasm may sometimes occur. In a few cases, Bouin's fluid was used on account of the sharp chromosome plates which it is said to give, though no improvement on the dichromate fixative could be observed.

Sections were cut at thicknesses ranging from 2 to 8 μ on a Leitz-Minot microtome. The most satisfactory stain for general purposes was the standard Heidenhain iron-haematoxylin, though Bolles Lee's (7) recent technique for rapid staining and differentiation was used in a few instances, and Ehrlich's haematoxylin was employed for temporary preparations. Gentian-violet (Gram) gave a few very satisfactory preparations of multipolar stages. Sections were in the main mounted in euparal, which seems to give sharper definition than Canada balsam, with slight loss of depth of focus due to its lower refractive index (1·483).

On the whole, the material was rather capricious as regards fixation, and a large number of buds had to be cut to secure a representative series of nuclear stages, though little difficulty was experienced in obtaining crisply stained and well differentiated preparations in properly fixed material. Several different stages can usually be found in different anthers of the same bud; such variation is greater in the winter months than in the summer, and November buds show all stages from synapsis to tetrad formation.

CYTOLOGICAL HISTORY.

The Archesporium.

In *Gossypium*, as in other species of the Malvaceae, the bilocular reniform anthers are arranged in typical looped 'festoons' on a staminal column, the lowest five being theoretically sterilized to form petals. In the earliest stage of the development of this column the festoon arrangement can be clearly distinguished, but as the anthers are carried up by the staminal column they can still be recognized as forming five double vertical bands of primordia; with further extension the arrangement becomes obscured, the anthers forming a densely packed layer on the surface of the column, though in cross-section the five double rows can still be seen. Owing to this packing of the anthers their natural orientation is obliterated, and in cross-sections the loculi will be cut at every possible angle.

The first differentiation of the anther primordia occurs when the bracteoles are sufficiently developed to meet at the bud apex and form a protective sheath. A true epidermis is first formed, increasing by lateral division and persisting in the mature anther. The archesporium proper arises from the subepidermal layer, localized areas of which divide to form three well-marked zones of cells, the innermost giving rise in due course to the pollen mother-cells. The morphological value of the archesporium is low, and it is almost impossible to distinguish the particular subepidermal cells from which these layers are formed, or to follow the process of nuclear division at this stage, on account of the small size of the nuclei and the large somatic number of the chromosomes; but numerous well-defined spindles and plates can be seen, showing the planes in which cell-division is occurring.

The Premeiotic Division.

The cells in the third layer differentiated by the archesporial divisions enlarge rapidly, while the surrounding layers continue to divide, and now appear in longitudinal section of the loculus as a definite band of tissue; the cytoplasm is dense and granular, and there are no vacuoles. When they have attained a diameter of about 14 μ they put in one final division, the premeiotic, from which they emerge as pollen mother-cells.

Owing to the rapidity and the fugitive character of this division, together with the large number of chromosomes and their small size, it is difficult to determine the exact sequence of events at this stage. The most striking feature is the definite increase in the amount of chromatin which takes place in the prophase; this, as in the case of *Primula* investigated by Miss Digby (8), appears to be produced by a process of budding from the nucleolus, which shows a very marked reticular-alveolar structure. During the earlier part of this phase the nucleolus often appears to be held in place by one or two bands of achromatic substance, which cross the nucleus diametrically (Fig. 1, Pl. XI); when a complete nucleus is found in a thick section, a characteristic 'cross' is seen. As the nucleus enlarges, more and more chromatin beads are seen, strung out on a fine achromatic reticulum which simultaneously thickens and becomes more visible (Fig. 2). The chromatin joins up into a continuous looped spireme, which fragments in diakinesis, the segments (in which no split can be discerned) becoming shorter and thicker (Fig. 3). There is no evidence of triple spindle formation, such as occurs later in the meiotic division. The nucleolus in prophase keeps its size, but becomes progressively less chromatic and finally disappears at the diakinesis (Fig. 4).

In the metaphase (Fig. 5) the chromosomes form a thick plate, and in diaster the individual dyads can be seen as short rod-shaped bodies, of the same shape as those of the root-tip mitoses, and markedly different from the coccoidal form which they assume in the meiotic and post-meiotic divisions. Spindle-formation occurs with faintly visible polar differentiation. There are no signs of the peculiar thread-ring structures described by Balls (3) in both somatic and haploid cells.

In the anaphase (Fig. 6), the polar bodies become temporarily more pronounced; the chromosomes, as they pass up the spindle, may show the usual V- and Y-forms, though these cannot readily be resolved. There are usually several laggard chromosomes, and in these cases it may occur that two or more are seen joined end to end and passing up the same fibril; this would seem to point to an incomplete separation of individuals in diakinesis.

In telophase (Fig. 7), a slight condensation of the chromosomes occurs before the first sign of the inception of the partition wall in the phragmoplast, but with the formation of the nuclear wall the chromosomes separate from one another (Fig. 8) and, as far as can be distinguished, join up to form a continuous spireme by a fresh fusion of the chromatic substance. As the spireme loosens out, the chromatin segregates into small beads along it, while at the same time one or two small karyosomes appear (Figs. 9, 10).

The nucleus then passes into the resting stage, and the beaded spireme moves outward to the nuclear periphery, while the chromatin is apparently resorbed by the nucleolus, which increases rapidly in size; if two nucleoli are present, fusion apparently takes place, since only one persists. The structure of the nucleolus is obscured by its greatly increased affinity for stains, but there are distinct signs of alveolation (Fig. 11). A few small beads of chromatin remain in the achromatic reticulum during the resting stage.

Meiotic Prophase.

The first indication of the onset of the reduction division is given by the separation of the cytoplasm of the pollen mother-cells from the surrounding tissue (though in most cases the cytoplasm continues to adhere to the mother-cell partition walls), so that they swim more or less freely in the locular cavity (Fig. 1, Pl. XIV). This appears to be due to a definite contraction of the cytoplasm; it was observed with all the fixatives used, and has been recorded by both Balls and Cannon. No reference to a similar contraction in other material is forthcoming, except in the case of *Syringa*, which Juel (9) considered an abnormality.

Simultaneously with this contraction, the tapetal layer begins to divide; in many cases no wall is laid down between the new nuclei, with the result that many tapetal cells are binucleate. As in other plants, the nuclear divisions are very irregular, and many abnormalities can always be found in the tapetum of otherwise normal anthers.

The first sign of renewed nuclear activity is found in the thickening and proliferation of the achromatic reticulum, while the sparse chromatin beads already present enlarge and become more prominent (Fig. 12). The achromatic reticulum develops rapidly as the cell enlarges, filling the nuclear cavity with its ramifications (Figs. 13, 14), while the chromatin beads break up into smaller granules which pass out into the new network, where they mark the intersection of the strands. At this stage signs of pairing become evident, though it is not clear how far this parallelism really exists and how much is due to the superposition of strands at different depths in the nucleus.

Synapsis.

The synaptic contraction comes on rather suddenly, and it is difficult to find a full series of intermediate stages between the fully developed reticulum and the synaptic knot. The first indication of approaching synizesis is given by the loosening away of the leptonema from the nuclear wall on the opposite side to the point of contraction, with a slight massing of the reticulum about the latter (Fig. 15). In complete synapsis the knot is apparently bounded by the strands which had previously been peripheral. Slight changes can be made out in this bounding layer, in thin sections, during synapsis, but it is impossible to resolve the centre of the knot; no loose ends or loops can be seen in knots which have not been touched by the knife. The nucleolus lies to one side of the knot and partially embraced by it (Fig. 16, Pl. XI and Figs. 1, 2, Pl. XIV).

In the loosening-out stage the knot becomes less granular and more fibrillar in appearance. Loops appear at the edges, in which chromomeres and a longitudinal split can be made out, pointing to the fact that syndesis has occurred, and that the spireme is now zygotene. The nucleolus is pushed out of the loosening knot, and lies caught up in the looped spireme (Figs. 18, 19, Pl. XI); its structure is now much more homogeneous, and in most cases no alveolation can be observed when the loosening is complete, nor has any chromatin budding been observed from this stage onwards. The spaces in the synaptic knot are still occupied by an achromatic substance, which disappears as the loops emerge (Figs. 3, 4, 5, Pl. XIV).

In view of the importance of the changes which take place during synapsis, every effort has been made, by varying staining and fixation, to obtain preparations in which details of the changes in the synaptic knot might be observed. Synapsis is here a phase of relatively long duration, since it is possible to cut buds in which every nucleus shows close synizesis, whereas in other buds, both younger and older, several different stages may be observed simultaneously. It has not been possible to follow more than the changes which take place at the extreme edge of the knot, however, even in the thinnest sections, and at the extreme limit of microscopic resolution.

The following tentative observations were made:

1. At the completion of the contraction the knot is bounded by the leptonema, which was originally peripheral to the nucleus.

2. The achromatic substance condenses to form a sponge-like reticulum, the spaces of which are occupied by a hyaline plasma; the chromatin is precipitated in this plasma in the form of fine granules, which are presumably free to move in the interspaces of the achromatic reticulum.

3. The synaptic knot expands slightly, and the achromatin takes on the form of a single tangled thread, with no sign of a split.

4. The chromatin granules are redeposited from the hyaline plasma on to the thread in a fairly even layer. They then appear to pass along the thread, forming rounded aggregations at irregular intervals.

5. The thread splits between these aggregations of chromatin, and as the split extends they pull apart into apparently equal portions or chromomeres.

The theoretical aspects of synapsis have been considered in some detail by holders of the parasynaptic theory of conjugation; under their scheme, the association of parallel threads before and during synapsis represents the pairing of entire chromosomes to form *bivalents*, which after much shortening and thickening are separated in the heterotypic mitosis, during the anaphase of which (or earlier in the case of chromosome tetrads) the split that is to function in the homoeotypic mitosis makes its appearance (Sharp, 10). Under the telosynaptic theory of conjugation the splitting of the thread represents the division of *univalent* chromosomes along the line which will again become evident in the heterotypic anaphase and the homoeotypic mitosis; this splitting may have had its origin in the pre-meiotic telophase. The *bivalent* chromosome is in this case formed by the association in pairs (at first end to end in the spireme, but later side by side) of segments of this split spireme at the time of the second contraction.

Conjugation in *Gossypium* is clearly of the telosynaptic type, as can be seen from the later behaviour of the spireme, and this observation seems fully confirmed by Cannon's account (2) of the formation of the chromosomes, published, however, before Farmer and Moore (11) had given the first definitive account of the process, or Farmer (12) had emphasized the essential differences between the telosynaptic and parasynaptic theories. The theory of the telophasic splitting, found by Miss Digby in *Galtonia* (13), *Primula* (8), *Crepis* (14), and especially *Osmunda* (15), and by Miss Fraser in *Vicia* (16), here lacks direct confirmation, though such splitting is thought to occur in the meiotic telophases.

In Miss Digby's description of the synapsis of *Osmunda* (15), in which it can be observed with exceptional clearness, it appears that the thread structure of the half univalent chromosomes is never obscured while the association of the threads and their sorting out is effected, nor is there any change in the distribution of the chromatin along them. Considerable divergence of opinion exists as to the nature of the chromomeres. Strasburger (17, 18) held that these are complexes of the 'pangens' of de Vries, interchanged during conjugation. Allen (19), in an account of the parasynapsis of *Lilium*, holds that the fusion of the leptotene threads involved the fusion of the chromomeres, which he took to be composed of still smaller chromatic elements, the resplitting of the pachytene thread being started by the redivision of the chromomeres. Gregoire (20), on the other hand, denies the individuality of the chromomeres, which he considers merely as thickened portions of an unevenly stretched thread: Wenrich (21), however, has found in the grasshopper *Phrynotettix* that in any given chromosome the chromomeres show a remarkable constancy in size and arrangement in different cells and even different individuals. In the present instance the precipitation and redisposition of the chromatin granules on the univalent thread would seem to agree with the observations of Allen, with the exception that the split is initiated not in the chromomeres but in the thread or univalent spireme. It is obviously impossible, in view of the length of the spireme and the large number of chromosomes, to identify the arrangement of the chromomeres. In the majority of preparations the doubleness of the thread cannot be made out except in short lengths, which can be found in all stages up to the second contraction, and even later. There is no evidence that any true fusion of the threads takes place, though the closely associated halves may appear at first sight as a single thick thread; at the same time there is nothing to prevent the formation of the 'chiasmatypes' of Janssens (22), with their important bearing on genetics. The loosening out of the univalent spireme from the synaptic knot is very much more gradual than the previous contraction, and agrees very closely with the process as reported in *Primula floribunda* by Miss Digby (8). The loops emerging from the knot are for the most part obviously univalent, though occasional portions are twice as thick as the remainder, showing marked twisting, with frequent longitudinal fission. This points to a certain amount of chromosome association already having taken place in the synaptic knot, with precocious formation of bivalents, though it is usual for this to occur only in the later second contraction.

As the nucleus passes into the well-known 'open spireme' stage (Figs. 20, 21) the looping becomes more and more accentuated until the knot is entirely unravelled and the loops have reached the periphery. At points of contact where there is anastomosis of the univalent strands there is always a chromatin bead or swelling (Figs. 22, 23). In some nuclei there is evidence of considerable tension on the strands which cross the centre; they are drawn out quite straight, and all signs of splitting are obliterated (Figs. 8, 9, Pl. XIV).

Examination of serial sections of open spireme stages shows that the spireme is quite continuous; the loops pass freely through and round each other, and there is very little anastomosis. In this condition there are no signs of any parallel arrangement of the strands, except for occasional bivalent lengths of thread. The height of this condition is reached when the whole of the spireme has passed out to the periphery and there are no longer any strands crossing the centre of the nucleus (Fig. 24, Pl. XII).

With the formation of the hollow spireme the structure of the cytoplasm begins to show marked changes. The nucleus moves from the side of the cell towards the centre, and the dense and rather granular cytoplasm grows progressively more reticulate in a radial pattern, increasing in density centripetally (Figs. 11, 13, Pl. XIV).

The hollow spireme must not be regarded as a condition of nuclear equilibrium; it appears that there are continual changes taking place in the arrangement of the loops, marked by an increase of the amount of anastomosis, and a general thickening of the whole thread, leading up to the second contraction of the spireme, in which the univalent chromosomes, united at their ends, are brought into the bivalent condition by the formation of loops.

Second Contraction.

The onset of the second contraction is extremely variable, and in a few cases it has been observed that it is omitted entirely, the nucleus passing direct from the open spireme to the segmentation of loops. In general, however, the loops of the open spireme withdraw from the periphery of the nucleus to one side (Figs. 2, 5, and 10, Pl. XIV); the spireme grows thicker and shorter, staining more deeply, and the chromomeres and chromatin beads disappear. At the height of the contraction the spireme can be seen as a tangled thread at one side of the nucleus, very much twisted and looped, but with none of the obliteration of structure which is characteristic of synapsis (Fig. 26, Pl. XII). The nucleolus (which already is becoming markedly less chromatic in its staining reaction) lies outside the contracted thread, but connected with it by two or more strands, and the whole occupies rather more than half the nuclear cavity. At the same time the cytoplasm shows an increase of the radial reticulation already referred to, and the formation of the typical perinuclear zone is begun.

As the spireme comes out of the second contraction it is evident that a very decided contraction and twisting has occurred and is still continuing, with the formation of a 'strepsinema' (Figs. 27, 28). Careful examination of these twisted portions reveals the presence of very fine achromatic strands uniting adjacent threads in a ladder-like form, and as soon as the loops of the twisted thread reach the nuclear wall segmentation of the twisted loops commences. The segmentation and condensation of the spireme into chromosomes has been described by Cannon (2), though his account has been questioned by Balls (3); from the description and the figures presented it can be seen that the process agrees almost exactly with the present observations, with the sole exception that no ring-shaped chromosomes have been seen in metaphase, nor do they appear to have U-, V-, or X-shapes in anaphase.

As the loops shorten and contract they break away from each other and assume the form of irregular rings (Figs. 29, 30, 31). Conjugation has occurred telosynaptically, and each ring consists of two split (univalent) chromosomes arranged end to end: the split in the univalent halves may often be visible right up to the final condensation of the ring into the bivalent chromosome. Cannon's observation that 'in any nucleus the loops and rings are of a uniform size' has not been confirmed, except in so far that no marked separation into two groups of different sizes can be made out. As the central split is obscured by further contraction, and diakinesis commences, the bivalents become more uniform in shape and size.

Diakinesis.

The phenomena of diakinesis and spindle formation are intimately connected with the perinuclear zone already referred to. This zone appears to be typical for microspore division in *Gossypium*, and is possibly characteristic of the Malvaceae in general: it is found in both the meiotic heterotype and homotype mitoses, but not in the premeiotic or somatic divisions. The formation of such a perinuclear zone and its bearing on the development of the spindle structures has been studied in great detail by Miss Byxbee (23) in *Lavatera*, and has been observed also by the writer in *Althaea.* Cannon makes reference to records of such zones in *Lilium* by Mottier (24), and in *Larix* by Belajeff (25), but these on examination seem scarcely comparable. More allied conditions have, however, been recorded by Juel (26) in *Hemerocallis*, by Hus (27) in *Cassia*, and by Mottier (28) in *Staphylea*, while similar structures have been figured in a number of other plants, and reference should be made to the recent work of Taylor (29) on the genus *Acer*.

Up to the point of diakinesis the process in *Gossypium* agrees closely with that described in *Lavatera*. The cytoplasm in early prophase is of a normal granulo-reticular structure, slightly looser at its periphery than round the nucleus, but fairly homogeneous in structure throughout the cell. Shortly before the nucleus passes into open spireme the cytoplasm appears to segregate into two constituents, the one granular and the other fibrous, the former having a faintly greater reaction to cytoplasmatic stains; and while the fibrous component loosens out into an irregular meshwork radiating outwards from the nucleus as centre, the granular constituent passes inward towards the nuclear wall, to form a dense layer, the structure of which cannot be resolved accurately, but which appears to be very densely reticular. In *Lavatera* this zone shades off gradually into the radial-reticulate meshwork, but in *Gossypium* the transition is more abrupt; in either case a certain break-down of the regularity of the radial reticulum occurs when the granular zone is completely formed (Fig. 14, Pl. XIV).

From Miss Byxbee's account it appears that spindle formation takes place in the following manner: The meshes of the network, close to the nuclear wall, pull out in a direction parallel to the wall, forming a felt of fibres about the nucleus, while the granular constituent of the cytoplasm collects in a wide, dense zone about the latter. This perinuclear zone remains quite distinct from the nuclear wall, and a mass of linin fibres forms in the intervening space, penetrating into the nuclear cavity on the breakdown of the nuclear wall at one or more points. These fibres, and others formed within the nucleus, together form a complex in which the chromosomes are distributed, and from it a multipolar spindle is projected; two of the cones of this spindle become more prominent than the others, which they finally absorb, thereby forming the bipolar spindle. The spindle has sharply pointed ends, which appear to be inserted in the perinuclear zone, and in early telophase there are traces of granular radiations from the poles, faintly suggestive (in the figures) of centrosomes. In the homotype division the process is repeated more or less exactly.

The chief point of difference between the spindle formation in *Lavatera* and in *Gossypium* is that in the latter there is no separation between the nuclear wall and the perinuclear zone. As the chromosome loops condense and pass out to the nuclear periphery the nucleolus disappears and, shortly after, the nuclear wall is absorbed. The chromosome rings can at this stage be seen partly embedded in the perinuclear zone, which begins to take on a distinctly fibrillar structure in the neighbourhood of each chromosome mass, the fibrillae radiating outwards through the zone. At the same time, other fibrillae can be seen uniting the various masses (Figs. 32, 33, Pl. XII). Here, as in *Lavatera*, it is apparent that the fibrillae are from two different sources, the radiating fibrils originating cytoplasmatically from the perinuclear zone, and the connecting fibrils probably from the nuclear linin, increased by the absorption of the nucleolus, which has been held by several observers to act as a reserve of fibrillar material (Strasburger (30, 31) and Eisen (32)). A similar stage has been described by Taylor (29) in the microsporocyte of *Acer Negundo.*

It is believed that the migration of the chromosome masses from the periphery to the centre of the nucleus is brought about by the contraction of the connecting fibrils, with the simultaneous elongation of the radiating or spindle fibrils (Fig. 15, Pl. XIV). (Traces of the connecting fibrils can still be seen in diaster as a fine reticulum uniting the chromosomes.) The whole body of the chromosomes in diakinesis passes evenly and simultaneously to the centre of the nucleus, where at first they form a dense and almost spherical aggregation (Fig. 34, Pl. XII, and Figs. 16 and 17, Pl. XIV), though an occasional laggard chromosome may follow at some distance, and the fibres of the multipolar spindle are evenly distributed over the whole nucleus as radii from this centre.

In the transition from the multipolar to the bipolar condition (Figs. 35–8, Pl. XII) there does not appear to be any absorption of these fibres as described in *Lavatera*, but rather a movement of the peripheral ends of the spindle fibres in the perinuclear zone towards two opposite poles, and as the fibres pass round towards these, quadripolar and tripolar stages may be found. Simultaneously, the chromosomes are pulled round from the spherical aggregation into the typical flat plate or diaster of the metaphase. Traces of the connecting fibrils can still be seen in diaster as a finereticulum uniting the chromosomes (Figs. 42, 43, Pl. XII, and Fig. 21, Pl. XIV).

The Meiotic Division.

It is difficult to distinguish with certainty the point of insertion of the spindle ends in the perinuclear zone and their attachment; the spindle is usually sharp-pointed, and in no case has there been any evidence of the presence of a thread ring uniting the ends of the fibrils as described by Balls. In good preparations there are definite indications of a very finely reticulate substance in the space between the spindle and the perinuclear zone, particularly in late anaphase, which possibly corresponds to the karyolymph described by Devise (33) in *Larix* and Miss Nothnagel (34) in *Allium*. The spindle ends frequently at points which are not diametrically opposite on the perinuclear zone, and in such a case a curved spindle results.

The chromosomes in typical diaster are coccoid in form, rarely showing a faint line of fission, and their number can be counted as 26, though two chromosomes appear to be markedly larger than the remainder; this question will be discussed in greater detail below.

The first appearance of the commencement of the anaphase is indicated by a ring-shaped constriction round each chromosome, and as the two halves are pulled apart the ring forms figured by Cannon may sometimes be seen. The daughter chromosomes in early anaphase are extremely irregular and even angular in shape, and in many cases three and even four fibrils can be seen attached to each, the chromatin being drawn out to a slight point at their insertions. As the chromosomes are further drawn out there may be a further separation of the halves of the univalents, indicating the division which will take place in the succeeding homotype mitosis. This separation is, however, extremely irregular, and occasionally the bivalent chromosome is pulled out into a line of about six irregular masses of chromatin (Fig. 44, Pl. XIII). As the univalent chromosomes pass up the spindle the irregularities of shape disappear and the coccoid form is reassumed; in many nuclei (as in the premeiotic division and in diakinesis) there are one or more chromosomes which lag behind the others (Figs. 39, 40), the bivalents in such a case sometimes remaining undivided when the other univalents are in late anaphase.

Some difficulty has been experienced in tracing the transition from anaphase to telophase, owing to the rapidity with which the former takes place. The first sign of the telophase commencing is seen in a slight decrease of the density of the perinuclear zone, together with a thickening of the spindle fibres, which bulge out between the daughter nuclei into a barrel-like figure. At the same time there is a distinct change in the spindle ends, which, from a definite pointed aggregation passing into the perinuclear zone, retract centripetally, while radial fibres can be seen spreading from them inside the zone, though they have not been seen to pass into the cytoplasm as described by Cannon. The cytoplasm surrounding the nucleus loses its radial arrangement and becomes loose and ragged, contracting slightly, and the collapse of the outlying reticulum may give rise to thread-like thickenings parallel to its periphery.

The chromosomes meanwhile break up into granules; it is impossible to count their number, or to identify them in any way as prochromosomes, and the granules are next seen arranged in a light reticulum (Fig. 46), apparently passing directly into a thin continuous spireme which is double (Fig. 50), while a limiting nuclear membrane forms. In some of the preparations examined a very short resting stage was apparently interpolated, in which the chromatin seemed to flow along the linin reticulum, giving the appearance shown in Fig. 47. This stage, though figured by Cannon, is probably an abnormal condition; in most cases the chromatin aggregates again into beads of irregular sizes, arranged on linin strands which appear to be continuations of the fibres of the original spindle (Figs. 48, 49).

The reconstructed nuclei lie a short distance inside the points of the original spindle, and there are no indications of a cell-plate formation in the latter. The original perinuclear zone expands and gradually disappears, and simultaneously similar zones are formed within it round each of the daughter nuclei, commencing as soon as the nuclear wall is laid down. The formation of these zones is on the lines already described, and the cytoplasm in their neighbourhood again takes on the typical radial structure; there is evidence for believing that part of the zone arises direct from the original spindle fibres (Fig. 51).

The spindle and the remains of the first perinuclear zone persist for some time and may still be seen when the tetrad cells are rounding off.

A point of some interest at this stage is found in the degradation of the cell-walls separating the original pollen mother-cells, anticipating the break-down of the tapetal layer at a later stage. These walls may be seen in all degrees of degeneration, with a typical granular appearance, though in no instance do they disappear entirely, traces still remaining when the pollen grains are completely formed; simultaneously a clear hyaline layer is deposited round the periphery of the cytoplasm (Fig. 23, Pl. XIV). The nature of this layer is obscure, and it was thought at first that it might consist of the 'callose' described by Mangin (35) in similar conditions. It is not soluble, however, in sodium hydroxide, nor does it stain with Mangin's corallin solution; it stains deeply with orange G, and gives a deep violet colour with Delafield's haematoxylin. It is more probable, from the rôle that this hyaline layer plays later in the separation of the pollen cells and the formation of their walls, that it is a complex of a mucilaginous consistency formed partly by the degradation products of the parietal walls and partly by a secretion of the cytoplasm itself. There is no evidence of any secretion from the tapetum at this stage other than the normal tapetal fluid in which the pollen cells are presumably floatin

Homotype Division.

In material which shows resting stages between the heterotypic telophase and the homotypic prophase, the chromatin passes from the reticulated or the beaded state into the double spireme already mentioned, and segmentation takes place without any looping of the thickened thread, the dyads forming by simple condensation and passing direct to the centre of the nucleus, where they form the usual plate. The process by which this is effected has not been fully observed, but there are signs of a multipolar spindle of the type already described. In some of the preparations made when the plants were in very active growth it appeared as if the chromosomes were passing direct from early telophase, without any change of form, to the homotype metaphase, no intermediate stages being found, though this may have been due to the rapidity with which such changes were proceeding.

As the chromosomes split and pass up to the poles, their outline is much more circular than in the heterotype division, and they do not appear to have the short rod form described by Cannon. No further split is visible at any stage of the anaphase, nor have any cases of the stringing out of the chromatin on the fibril been seen as in the meiotic anaphase. The telophase proceeds as before; the chromosomes disintegrate into granules, which recombine into irregular beads in the resting nucleus, and one or two small nucleoli are formed. At the same time, a new perinuclear zone is laid down which persists during the formation of the wall of the pollen grain. This zone is very much more granular than the previous rings, and expands gradually until in the mature grain it lies at the boundary of the cytoplasm; the separation of the zone from the nuclear membrane occurs shortly after the separation of the pollen cells from each other and before the wall is begun.

The pollen tetrads are rather irregularly arranged, generally in tetrahedra, though occasionally all four nuclei are found in a flat plate. Cytokinesis is brought about by furrowing, somewhat on the lines described by Farr (36) in *Magnolia*, although there are not always traces of the commencement of the constricting furrow after the first mitosis,[1] and the existence of the six spindles connecting the four nuclei each to each, a conspicuous feature of the process in *Nicotiana* (37), is only faintly discernible. Small furrows or constrictions appear at the periphery of the cytoplasm, midway between each nucleus, and spread inwards till they meet at the centre, cutting the four nuclei apart from each other; the larger spindle fibrils offer a certain resistance to the advance of the furrow, which appears to pass round them, but they eventually retract and take no further part in the cell processes. The hyaline layer already described closely follows up the advancing groove, flowing in from each side, but the adjacent surfaces do not fuse, and a definite partition line is left, along which separation eventually occurs (Fig. 15, Pl. XIV, and Figs. 53–5, Pl. XIII).

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[1] These can be seen more distinctly in Upland material.

After the separation of the pollen nuclei, a slight contraction of their cytoplasm takes place to form some six or eight indentations in the periphery (two or three visible in section), and at these points presently are formed an equal number of extranuclear bodies which are apparently concerned with the formation of the germ pores of the pollen-grain wall, since they are always found opposite these pores at a later stage; they are arranged evenly round the protoplast and increase but slowly in size. Their composition is obscure; they stain vigorously with orange G, 'Heidenhain', and 'Delafield', but the coloration with the last two stains is considerably more brown than that of chromatin. At a later stage they appear to pass from the cytoplasm into the pollen wall, where they apparently inhibit its growth in thickness above them, and they finally disappear at the same time as the hyaline plasma in which the wall is laid down.

A full account of the formation of the elaborate wall of the pollen grain is beyond the proper scope of this paper, but the following details are available:

After the separation of the tetrad nuclei, the hyaline layer round each increases considerably in width (Fig. 56), and a rather thick wall is differentiated at its inner surface. The spines of the wall, the perispore proper, are developed in the hyaline layer outside the thick wall, the mamelon on which each spine rests arising at the surface of the wall, though not till the spines have been completely formed. At the same time the wall itself is differentiated into three concentric zones: an exine or exospore, at the surface of which the mamelons are produced, a transparent mesospore, which later shows radial lamellation into 'rodlets', and a denser endospore. There are also indications of a very thin limiting pellicle at the surface of the cytoplasm, which is for the most part not in contact with the developing wall (Fig. 58).

During the formation and differentiation of the wall, the pollen grain is not spherical, but collapsed into an irregular form, largely concave (Fig. 57). Since only a limited area of the cytoplasm is in contact with the wall, this may be regarded as a further example of the anomalous growth of spore walls separated from their protoplasts described by Beer (38) in the Onagraceae and by Tischler (39) in sterile hybrids of *Mirabilis*. There is, however, some diminution of the protoplast during the differentiation of the wall, pointing to a certain expenditure of protoplasmic substance in its production. As the spines of the wall are formed the hyaline layer disappears, and can no longer be found when the process of wall differentiation is completed.

The tapetal layer remains intact until the hyaline layer is removed, when it breaks down to form a typical 'plasmodium' which flows in among the grains, the protoplast of which at once begins to increase in volume, until the whole grain is filled with a dense and rather fibrous cytoplasm, and its shape becomes spherical. Simultaneously with the irruption of the plasmodium the pollen wall becomes cutinized, taking on a yellowish colour and no longer staining with 'Delafield'. The pollen grain continues to increase in size until the anther bursts, the wall, now in contact with the protoplast except at the germ pores, increasing in area by intussusception without alteration in thickness, and shortly before the flower opens the nucleus divides again to form the generative and tube nuclei, the latter being slightly the larger of the two. No details of this division are at present available, though it appears that no perinuclear zone is formed in the process.

Thread Rings.

The material on which Balls based his theory of the thread-ring mechanism of nuclear division was a commercial strain of *Mit Afifi*, an Egyptian cotton of the *G. barbadense* type; it is therefore not unreasonable to use the material of the present investigation for comparison. The main outline of this theory may be quoted briefly. As the spireme loosens out from synapsis, it shows as a continuous thread in which are embedded rows of granules, each showing a distinct longitudinal bisection.

> 'The nucleolus next decreases in size, and the granules stain more darkly . . . the darkening is localized to those portions of the thread which correspond to the future position of the chromosomes. Except in these portions, the thread of the spireme is now split. Each of these clusters of darkened granules becomes . . . a bivalent chromosome. This chromosome is not, however, merely bisected as were the granules, but is also divided transversely to the axis of the spireme thread; four perfectly distinct chromatic areas are thus formed, being the univalent chromosomes which are to be distributed to the four microspores . . . the chromosomes are bunched together at one side of the nucleus, and there is no peripheral distribution. In this respect cotton differs from most organisms and from its own vegetative cells.' 'The two halves of the split spireme move apart from one another at the side where the chromosomes are lying, but this separation

does not affect the chromosomes. The chromosomes are isolated by the removal of the spireme halves, but not entirely, for continuity with the latter is maintained by thin filaments on either side. The insertion of these filaments (the young spindle fibres) in the spireme halves causes slight swellings which appear to the eye as black dots.

'From this point I propose to refer to the spireme halves as the "thread rings", retaining the term "fibre" for its usual subject.

'The two thread rings continue to separate, the fibres becoming longer and longer, until the part of each thread ring which bears the dots has moved to a pole of the nucleus. The dots are scattered round some 100° of arc, and the remainder of each thread ring forms an irregular tangle of granular cytoplasm. The looping of thread rings seems to be due to the fact that the circumference of the close spireme is greater than the circumference of the nucleus. This stage constitutes the multipolar spindle.

'The next event is the contraction of the dotted portions of the thread rings, bringing the dots nearer together, and forming the bipolar spindle of metaphase. The looped thread rings lying in the clear zone between spindle and granular cytoplasm are quite conspicuous; this was the observation which initiated the present research. It sometimes happens that a stray dot is not drawn up into the cluster at the pole as soon as it should have been; in this case the spindle fibre which ends in it remains at the side of the spindle, often slack and bent, instead of being drawn taut.

'The polar dots at the ends of the fibres have been noticed by other observers, but I can find no mention of their cross connexion by the threads.'

It has been shown in the present paper that the reduction division of the microspores of cotton proceeds along fairly normal telosynaptic lines, in agreement with the presentation of the facts first given by Cannon (with a few exceptions of minor importance), and it is perhaps unnecessary to analyse all the points in which the account of Balls differs. The two main discrepancies (leaving out of question the method of conjugation) are the supposed bunching of the chromosomes at one side of the nucleus, prior to diakinesis, and the existence of the loops figured by Balls between the perinuclear zone and the spindle. While the former may perhaps be explained by reference to the second contraction of the spireme, which is not mentioned in Balls' account (although the passage in Cannon's paper to which he refers certainly describes it), the question of the loops within the perinuclear zone is more complex. Achromatic ring figures of a sort have undoubtedly been seen from time to time during diakinesis, metaphase, and anaphase by the writer, not only in cotton (Fig. 59) but in *Allium*, *Crepis*, and *Dolichos*, but always in imperfectly fixed material, and no reference has been found to such rings in cytological literature.

Balls does not claim that the thread-ring mechanism is peculiar to the meiotic divisions, but states that it may be clearly seen in the somatic mitoses of the root-tips. Several hundred sections of root-tip material have been examined in an endeavour to observe thread rings, using various stains and fixatives, but with negative results, though all stages of somatic mitosis have been searched. In the somatic cells there is often a distinctly reticulate structure in the cytoplasm in which individual reticulations might by their size and prominence be taken for thread rings, though they may be seen in all parts of the cytoplasm at all stages of nuclear division, and particularly with the nucleus in the resting stage.

A large number of microspore heterotype and homotype spindles have been similarly examined under critical conditions in single and serial sections, with equal lack of success. Occasional well-marked rings have been seen, usually at one side of the spindle (Fig. 20, Pl. XIV). These might be explained as stray mantle fibres, but are more probably due to the fixation of the karyolymph in the form of a coarse reticulum. The nature of this reticulum and the fineness of its meshes varies according to the fixative employed. 'Flemming' and 'chromacetic' give a coarse network with some distortion of the spindle fibres, while with 'von Teleyesniczky' or 'Bouin' the reticulation is almost invisible. In one slide a similar small ring occurring near the plate in metaphase proved to be visible in four successive sections, and was apparently a cylindrical or spherical inclusion of some kind. Small rings can also be seen in badly fixed multipolar stages where, under Balls' theory, they should not be visible (Fig. 19, Pl. XIV). It is concluded that the appearances on which the thread-ring theory was based were due to imperfect fixation, under extremely trying working conditions, at a date when the full importance of the mode of chromosome conjugation had not yet been realized.

It must be pointed out, however, that the cotton plant, with its large number of minute chromosomes and its complex cytoplasmatic organization, is by no means a suitable subject either for the establishment of new cytological theories or for criticism of existing hypotheses. The structures in question are barely within the limits of effective visibility, and the possibility of subjective error is large.

Chromosome Numbers.

The chromosome number of Sea Island cotton, *Gossypium barbadense*, var. *maritima*, Watt, has not been previously determined. The type examined by Balls was commercial *Mit Afifi*, an Egyptian derivative of *G. barbadense* probably, though placed by Watt under *G. peruvianum*, Cav., and its chromosome number was stated by Balls as 20. Haploid material of *Mit Afifi* has not yet been available for examination, but a number of good chromosome plates have been found in transverse sections of the meristem of root-tips from seed of the commercial cotton. The chromosomes in the somatic cells are in the form of short rods, generally curved, and resemble those of similar cells in the Sea Island types very closely. Counting is a matter of some difficulty, even under the highest magnification, but the number can be stated with certainty as greater than 50, and has been counted as 52 on several occasions: it would appear that the number given by Balls, which would be 40 in the somatic cells, is an under-estimate.

Cannon, working with F_1 hybrid material of *G. barbadense* (commercial Sea Island, Constellation brand) × *G. hirsutum* (commercial Upland, Klondyke brand), found the chromosome number to be 28, though unfortunately neither of the parent types were studied cytologically.

Repeated counts of perfect plates in all the material examined show that the number of the chromosomes in all types of Sea Island cotton is more probably 26 than 28. The slight doubt arises from the heterogeneity of the chromosome size and shape, and it appears that there are two chromosomes markedly larger than the remainder, with a slight constriction which gives them the appearance of closely adjacent or 'clumped' individuals. Similar constrictions have been recorded in chromosomes of *Vicia* and *Najas* by Sakamura (40, 41) and in *Fritillaria tenella* by S. Nawaschin (42), and their bearing on the apparent chromosome number and its variation in individuals of the same species has been discussed by several writers, including Sakamura, Winge (43), and Tischler (44, 45).

There are difficulties in the counting of large numbers, where it is impossible to check by reference to profile views, and errors may arise in several ways: from the counting of a plate which is still in late diakinesis, with some of the chromosomes clumped or at different levels, giving too small a number; from counting an early anaphase, where some of the chromosomes have already separated; from the presence of laggard chromosomes which have not taken up their proper position, or precocious chromosomes which pull away in advance of the others. In all cases the serial sections above and below should be examined, particularly with thicknesses of less than 5 μ.

It is not possible to arrive at chromosome numbers in doubtful cases by a statistical treatment of the counts, on account of the peculiar nature of the errors of observation, and the decision must always rest on personal judgement. At the same time, the tendency to give a 'probable' number must be guarded against. Winge (43) has summarized this danger as follows:

> 'There is a general inherent inclination in the human mind, when dealing with numerical questions, to grasp at the "nice" figures. . . . The even numbers and highly divisible values have a certain attraction in themselves which is naturally increased when other considerations speak for their selection. Every cytologist is brought up to regard it as a fact that the chromosomes are halved before a division of the cell, and that the number of chromosomes is alternatively halved and doubled during the alternation of generations; there is therefore a natural temptation to find highly divisible multiples of 2 throughout. . . . A considerable number of incorrect

chromosome values have indubitably resulted, as many plants, either through the presence of an especially great number of chromosomes or by the minimal size or awkward situation of the same, have offered particular difficulties in determination of the number, and thus led the investigator to decide the point, albeit perhaps unwittingly, according to his personal estimate.'

It is often possible to check chromosome numbers by comparison of other species of the same genus. Winge (43), investigating the Chenopodiaceae, found the chromosome numbers of 11 species in 5 genera to be either 6, 9, or 18—i.e. 3 × 2, 3 × 3, and 3 × 3 × 2 respectively. In the Ranunculaceae the cardinal number appears to be 6; in *Crepis*, however, the cardinal number may be 3, 4, or 5, though Miss Digby (14) quotes the observations of Fraser and Snell (46) on constrictions as a possible explanation of the anomaly.

Unfortunately, the only chromosome numbers of the Malvaceae yet published are the two rather doubtful values for *Gossypium* (20 and 28) already quoted. These are both easily divisible multiples, and more attractive than 26, with its cardinal number of 13 as against their 5 and 7. Until further plates are available in other species of *Gossypium* and other genera of the Malvaceae it is evident that the comparative method is useless; at the same time it may be pointed out that there is no inherent improbability in the number 26, for in Tischler's (45) latest list a number of plants may be found with the cardinal number 13, and in the Aceraceae recently investigated by Taylor (29) three species have 26 chromosomes and two 13, although the latter does not appear to be the cardinal number for the genus.

Abnormalities.

In the first-generation hybrid investigated Cannon found a number of abnormalities, chiefly of the nature of amitotic divisions. These were not observed by him in greenhouse material collected in November and December, but occurred in material taken in the spring. Cannon expresses a doubt 'whether these abnormal divisions were due to cultural conditions, to the fact that the plant was a hybrid, or to both these factors with the added one that the flowers were the last to form on the plants'. Balls states that these irregular divisions can be found in all cotton plants if very late flowers or very early ones on ratoons are taken, and that they are not necessarily due to hybrid constitution, but might be provoked by the greenhouse culture employed.

In the very large number of buds taken for the present investigations at all times of the year and from plants in all stages of growth, including ratoons, there is no evidence whatever of amitotic division, either in the pure Sea Island strains or in commercial Upland varieties. This would point to some other cause than greenhouse culture for amitosis; bad culture, with extremes of temperature and widely varying humidities, tends to produce considerable bud shedding, but in these circumstances a general necrosis of the staminal column ensues, clearly recognizable in its earliest stages. It is conceivable that in the cases described by Cannon the amitosis was due to the presence of a lethal combination of genetic factors acting on the microsporocyte, though confirmation of this is lacking.

Such degeneration as was found in the material examined was of the nature of a localized contabescence, the contents of one or more loculi, in an otherwise normal anther, showing complete disorganization. The nucleus breaks down, and the chromatin is dispersed through the cytoplasm, which stains so deeply as to obscure all evidence of structure in many instances. Partly degenerate cells may show the nuclear wall still intact, but the chromatin irregularly dispersed in a coarse reticulum within it, while at the same time the cytoplasm contains chromidia-like bodies in large numbers throughout its substance.

This contabescence is still under investigation, but it appears that the normal secretion products of the cotton plant which occur in all the flower buds in isolated cells and lysigenous glands may, under certain conditions, be secreted in the spore protoplasts, where they interfere with normal development and cause abortion. Evidence of similar secretion has been found in aborted cells of the megaspore; for fuller details of these substances and their occurrence in all parts of the plant and in other species of Malvaceae, reference may be made to the work of Stanford and Viehover (47).

In addition to contabescence, it is noteworthy that in two instances an apparent diploidy has been observed in the first meiotic division, though it was not possible in either case to count the chromosomes seen in profile, and it is not known how the duplication arose. In one case the nucleus was in late anaphase, but the shape of the chromosomes was typically meiotic: in the second the chromosomes were in late diakinesis, but in both the amount of chromatic substance was apparently double the normal, and the nuclei were abnormally large. No evidence of a similar condition has been observed with any certainty at any other stage, however, and all the other nuclei in these two buds were normal.

SUMMARY.

The process of pollen formation in Sea Island cotton is described and figured.

The reduction division takes place along normal telosynaptic lines, and agrees on the whole with the process as described by Cannon in hybrid cotton plants.

Points of special interest occur: (*a*) in the perinuclear zone, which is apparently largely concerned with spindle formation in both homotype and heterotype divisions, but not in the premeiotic or somatic divisions; (*b*) in the manner in which the chromosomes are brought to the centre of the nucleus in diakinesis; and (*c*) in the method of tetrad division by furrowing.

The chromosome number is 26, with two chromosomes distinctly larger than the remainder.

Thread-ring structures which have been described in parallel material have not been found in well-fixed buds, but ring figures of a similar appearance occur in cells which show distortion by the fixative.

The writer wishes to record his thanks to Mr. H. Gunnery, who prepared most of the slides used, and gave in addition much very valuable assistance in the working out of the cytological history.

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EXPLANATION OF PLATES XI–XIV.

Illustrating Mr. Denham's paper on Microspore Formation in Sea Island Cotton.

All the figures were drawn with Zeiss large camera lucida under a 1/15″ Koristka semi-apochromatic immersion objective, N.A. 1·30, with Zeiss compensating ocular 18 (old notation), and Leitz aplanatic condenser of N.A. 1·40 immersed, except Figs. 34–43, 46–52, 58, for which Zeiss 2 mm. apochr. objective N.A. 1·40 was used, and Figs. 53–7 with Zeiss 4 mm. apochr. objective N.A. 0·95.

PLATE XI.

Fig. 1. Premeiotic division. Resting stage, large nucleolus (vacuolate) in achromatin bridle across nucleus.

Fig. 2. Increase of chromatin by budding from nucleolus.

Fig. 3. Nucleolus disappearing; continuous spireme.

Fig. 4. Spireme thickens and fragments into chromosome lengths.

Fig. 5. Metaphase of premeiotic division.

Fig. 6. Early anaphase, chromosomes rod-shaped.

Fig. 7. Commencement of telophase, with aggregation of chromosomes.

Fig. 8. Telophase. The nuclear membrane reappears, and cell-plate is formed.

Figs. 9, 10. Segregation of chromatin into granules on continuous achromatin spireme. Two karyosomes present.

Fig. 11. The karyosomes have fused into one large vacuolate nucleolus, and the nucleus appears empty except for a few chromatin granules at the periphery.

Fig. 12. The nucleus increases rapidly in size; the achromatin reticulum spreads through the cavity, with chromatin beads at the intersection of the strands. Nucleolus markedly alveolate.

Fig. 13. The achromatin reticulum increases and thickens.

Fig. 14. The reticulum is now apparently double in parts.

Fig. 15. Commencement of synaptic contraction. The reticulum pulls away from the nuclear wall. Nucleolus less alveolate, but still showing vacuoles.

Fig. 16. Close synapsis in its earliest stage.

Fig. 17. Synapsis. No trace of structure in the knot now visible. The nucleolus stains solidly.

Figs. 18, 19. The synaptic knot loosens out, and the spireme can be seen as double in a few places.

Fig. 20. The knot completely unravelled. The spireme is markedly double, and chromomeres can be seen, with beads of chromatin at intersections.

Fig. 21. Commencement of open spireme; chromomeres clearly marked.

Fig. 22. Open spireme. Section below nucleolus. Some doubling of threads visible.

Fig. 23. Anastomoses disappearing. Much apparent doubling, and chromomere lumps on thread.

PLATE XII.

Fig. 24. Completion of open spireme, no strands left in centre of nucleus.

Fig. 25. Commencement of second contraction, with marked thickening of spireme.

Fig. 26. Height of second contraction.

Fig. 27. Loosening out of thickened spireme, clearly double and twisted.

Fig. 28. Section through looped thickened spireme.

Fig. 29. Spireme segmenting into lengths.

Fig. 30. Condensation of spireme lengths to bivalent chromosomes. The nucleolus begins to disappear.

Fig. 31. Nucleolus almost gone. Clearly marked chromosome loops.

Fig. 32. The cytoplasm has taken on the typical radial structure, and the perinuclear zone has formed. The chromosomes are united by fibrils in this zone.

Fig. 33. The same stage as Fig. 32, a tangential section through the perinuclear zone showing fibrillar strands uniting all chromosome masses.

Fig. 34. The chromosome masses pass inwards to the centre of the nucleus, as the fibrillar band contracts, drawing the spindle fibres after them.

Fig. 35. Multipolar spindle from badly fixed material, with distorted spindle fibres and 'rings'.

Fig. 36. As the spindle fibres move round to the poles a tripolar spindle forms.

Fig. 37. Slightly later. A quadripolar spindle.

Fig. 38. Heterotypic metaphase. Some of the chromosomes beginning to pull out.

Fig. 39. Early anaphase; curved spindle with precocious chromosomes.

Fig. 40. Early anaphase.

Fig. 41. Anaphase, with one pair of chromosomes drawn out into rods.

Figs. 42, 43. Plates showing twenty-six chromosomes, united by faintly visible connexions. In each case two chromosomes are markedly larger than the others.

PLATE XIII.

Fig. 44. From two spindles in early anaphase. The chromatin is drawn out by the spindle fibres into irregular masses, suggesting the separation of the homotype chromosomes.

Fig. 45. Late anaphase.

Fig. 46. Early telophase. The chromatin is precipitated as granules on a very fine continuous spireme.

Fig. 47. The chromatin flows into a coarse reticulum; (only instance observed; possibly abnormal intercalated resting stage).

Fig. 48. Typical interstage. The chromatin forms rounded masses slung on achromatin strands in the direction of the previous spindle fibres. A new perinuclear zone forms round each nucleus.

Fig. 49. An interstage. A faintly staining karyosome is present.

Fig. 50. A homotype prophase.

Fig. 51. Homotype metaphase. The spindle fibres of the heterotype division can still be seen, and the remains of the original peninuclear zone.

Fig. 52. Two homotype plates. That on the left is slightly oblique; the chromosomes are passing into anaphase. The right-hand plate is in late diakinesis.

Fig. 53. Division by furrowing. The furrow is seen commencing at the interstage between the first and second divisions. The outermost (continuous) line marks the edge of the hyaline plasma, the dotted line a faintly differentiated zone at the edge of the cytoplasm.

Fig. 54. Pollen tetrad in tetrahedral arrangement. Furrowing has commenced at the edges, and has already reached the centre from below.

Fig. 55. Pollen tetrad in flat plate. An advanced state of furrowing.

Fig. 56. The tetrad completely separated. A dense wall is differentiated at the edge of the cytoplasm, and a number of deeply staining bodies have appeared in each cell.

Fig. 57. Formation of pollen grain wall in collapsed state. Hyaline plasma not shown.

Fig. 58. Section through the wall of mature pollen grain at a germ pore. The limiting pellicle is shown black.

Fig. 59. 'Rings' due to faulty fixation: an early meiotic anaphase.

PLATE XIV.

All the figures are from microphotographs taken with Zeiss 4 mm. apochr. objective N.A 0·95, Leitz aplanatic condenser N.A. 1·00 dry (3/4 cone), Wratten filters 45 and 62, Ilford ordinary plates. Figs. 4, 5, 6, and 7 taken with Leitz periplanatic eyepiece 6×, remainder with periplanatic 10×.

Fig. 1. Presynapsis. The upper cell shows the reticulum just pulling away from the nuclear wall; in the lower cell the contraction is well advanced.

Fig. 2. Synapsis.

Fig. 3. Loosening out from synapsis.

Figs. 4, 5. Later stages of loosening out.

Figs. 6, 7. Nucleus passing into open spireme.

Figs. 8, 9. Open spireme with gradual thickening of thread.

Fig. 10. Second contraction.

Fig. 11. Thick spireme loosening out, with twisting.

Fig. 12. Spireme commencing to segment.

Fig. 13. Segmentation. Perinuclear zone well marked, and cytoplasm showing coarse radial reticulation.

Fig. 14. Perinuclear zone and fine radial reticulation.

Fig. 15. Concentric fibrils uniting chromosome masses. First appearance of hyaline zone.

Fig. 16. Chromosome masses passing in towards centre.

Fig. 17. Multipolar spindle.

Fig. 18. Tripolar spindle.

Fig. 19. Two multipolar spindles from badly fixed material, showing distorted fibres and 'rings'.

Fig. 20. Heterotype spindle, slightly distorted. Note 'ring' at lower end, and others at extreme right of cytoplasm.

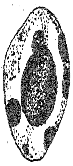
Fig. 21. Heterotype plate.

Fig. 22. Anaphase.

Fig. 23. Interstage. Hyaline zone well marked.

Fig. 24. Homotype division; chromosomes being drawn into centre of nucleus by contraction of fibrillar ring.

Fig. 25. Division of tetrahedral tetrad by furrowing.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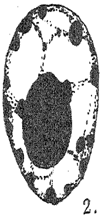
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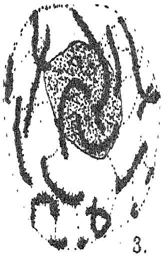
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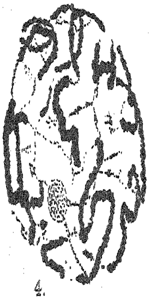
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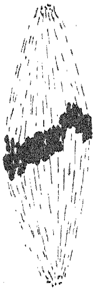
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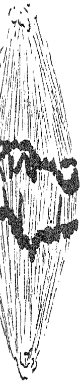
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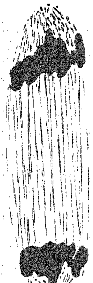
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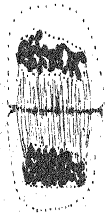
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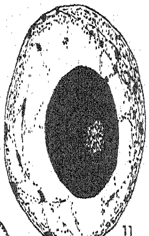
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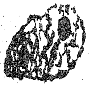
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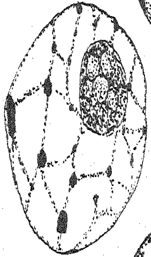
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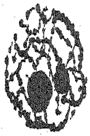
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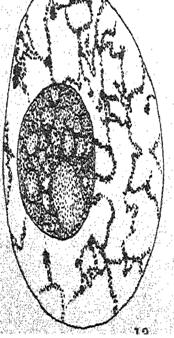

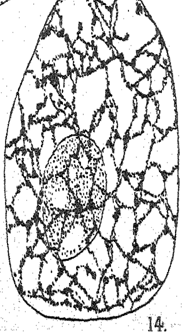
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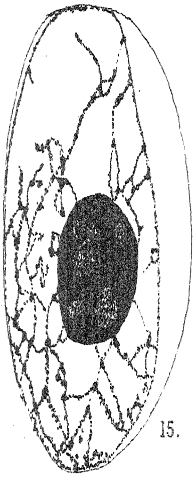
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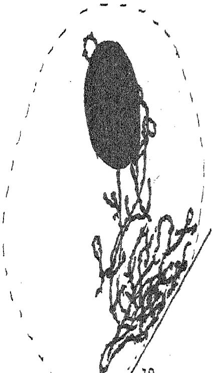
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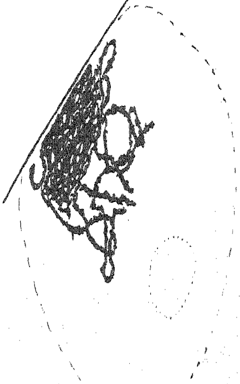
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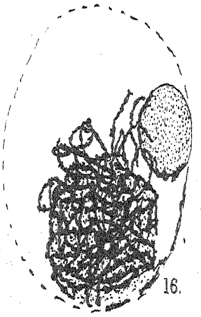
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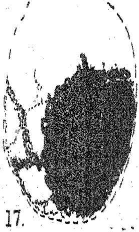
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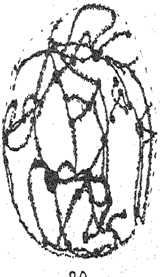
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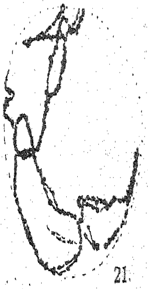
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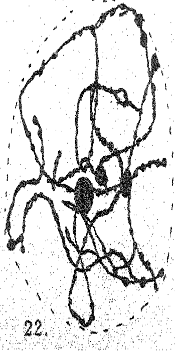
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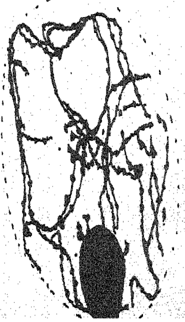

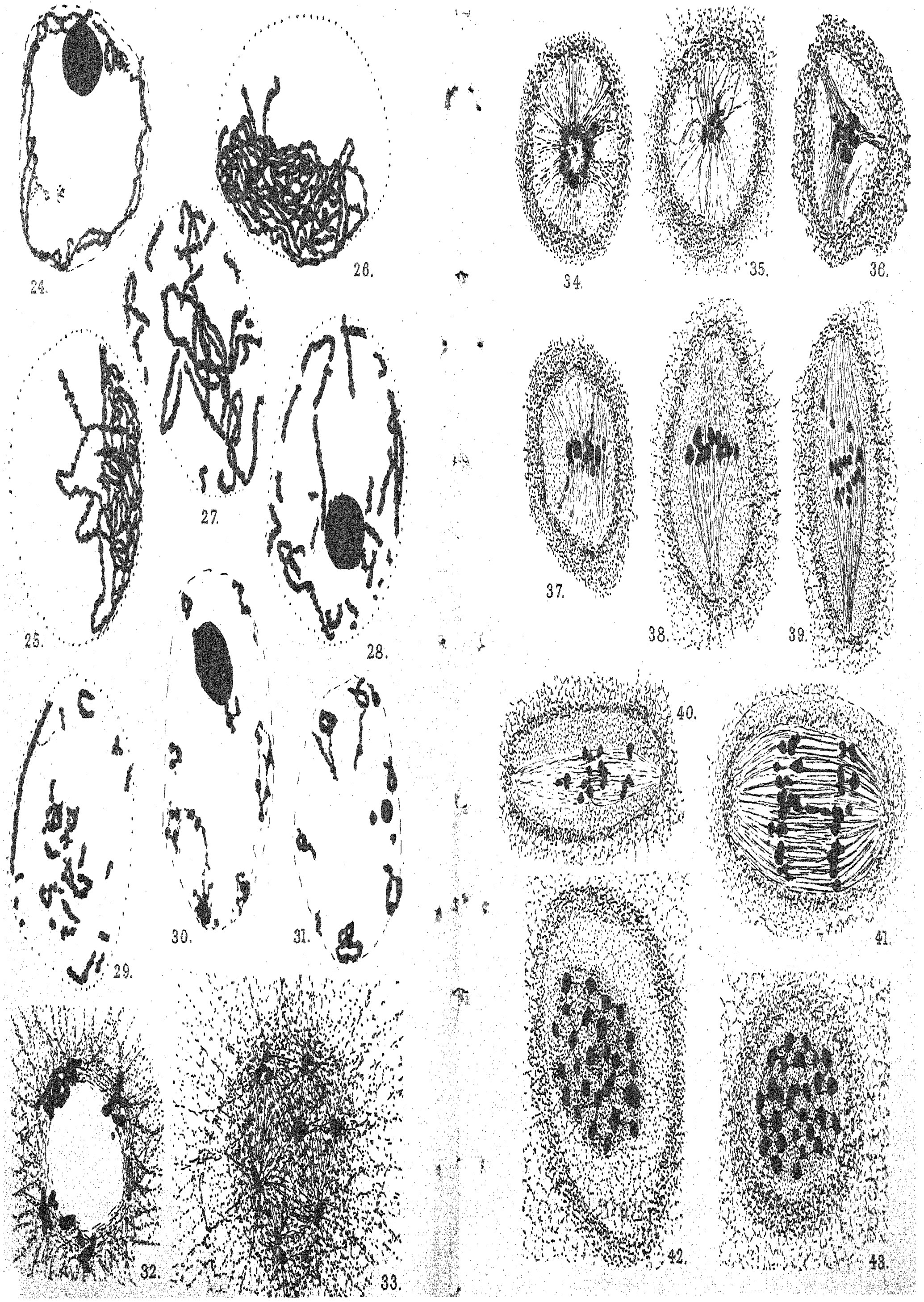
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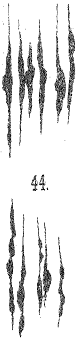
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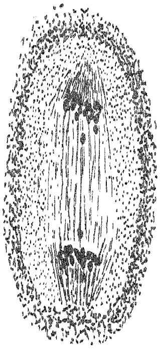
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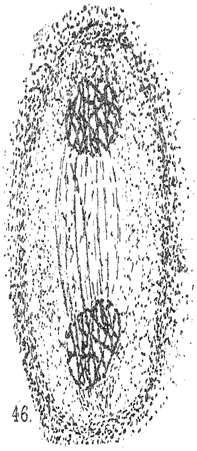
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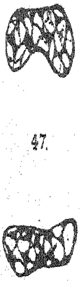
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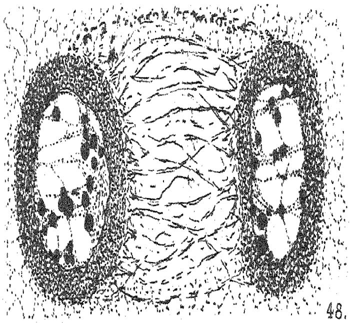
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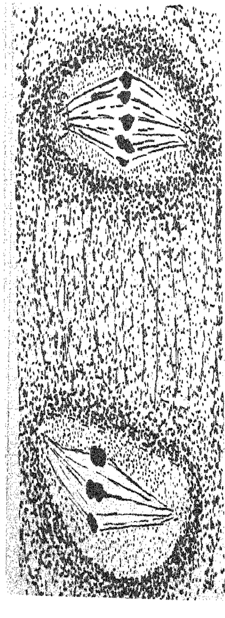

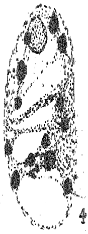
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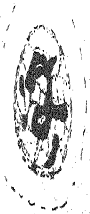
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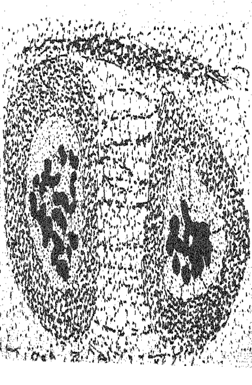

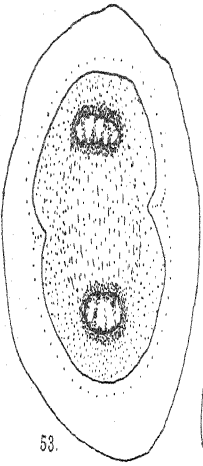
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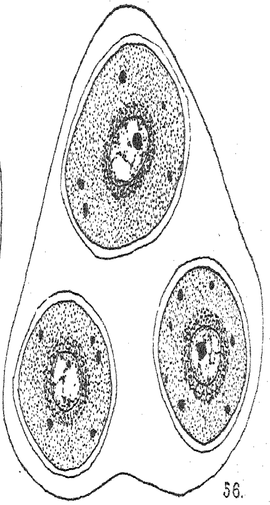
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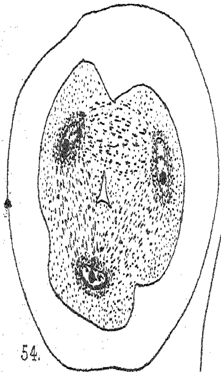
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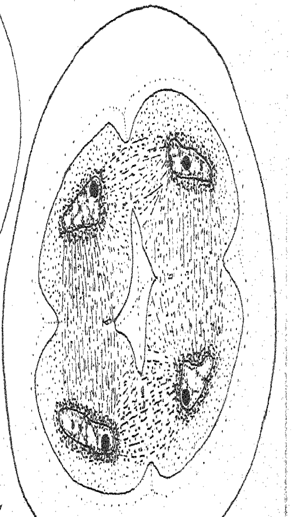
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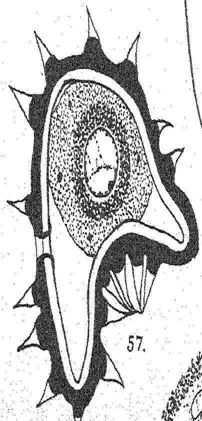
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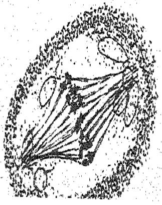

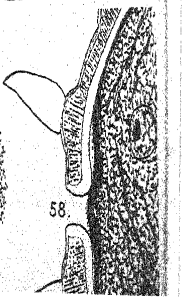
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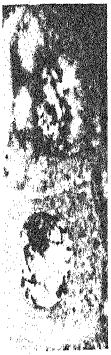

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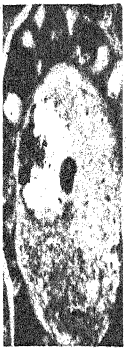

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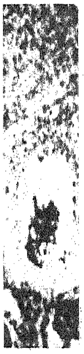

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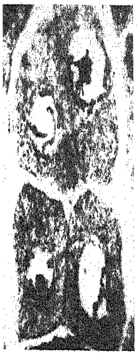

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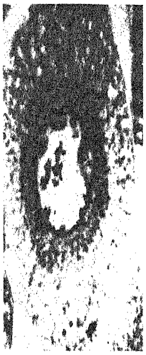

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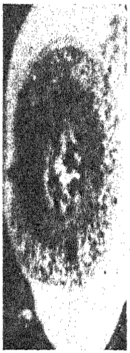

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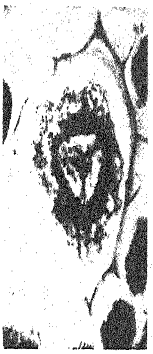

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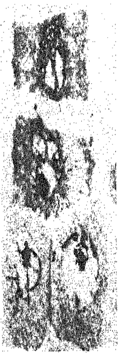

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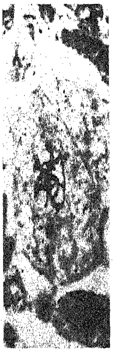

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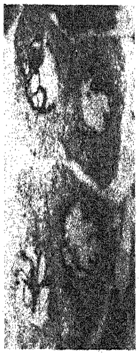

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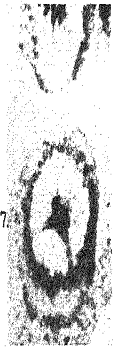

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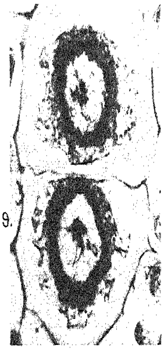

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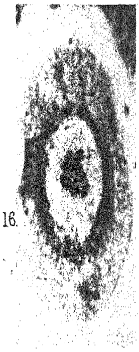

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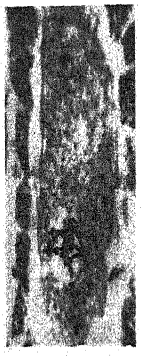

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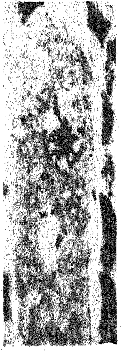

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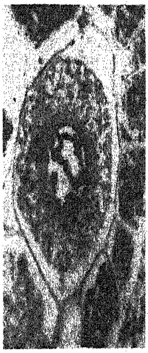

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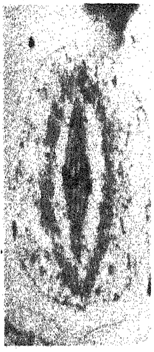

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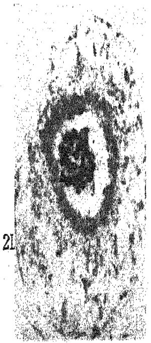

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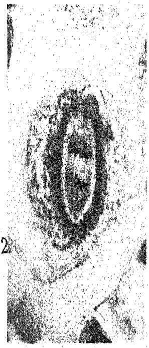

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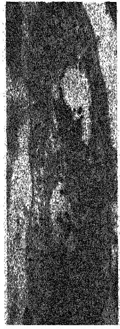

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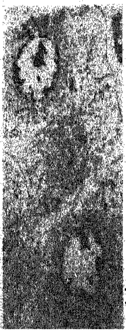

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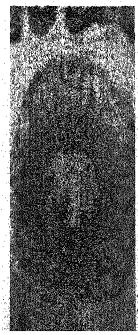

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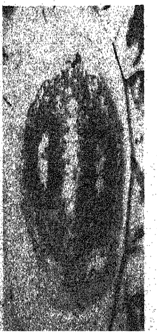

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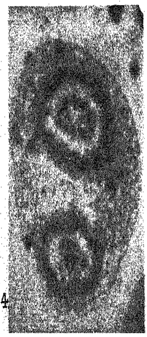

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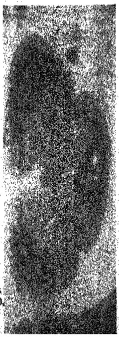

The Cytology of the Cotton Plant.

II. Chromosome Numbers of Old and New World Cottons.

BY

HUMPHREY JOHN DENHAM, M.A. (OXON.), F.R.M.S.

(*The British Cotton Industry Research Association.*)

With eleven Figures in the Text.

INTRODUCTION.

IN the first paper the cytology of pollen formation in Sea Island Cotton (*Gossypium barbadense*, var. *maritima*, Watt) was discussed in some detail. The writer is now in a position to give the chromosome numbers of the several types which have been cultivated in the experimental greenhouse of the Shirley Institute at Didsbury during the last three years.

The chromosome number of Sea Island Cotton has already been given as 26. It has now been found that Upland types give the same number as Sea Island in every case, but that Indian and Chinese cottons so far examined have 13 as their chromosome number. Detailed lists of the types examined cytologically are presented, with figures of chromosome plates of some of the main varieties; all the numbers should be read as haploid, unless definitely stated as diploid.

MATERIAL AND METHODS.

The methods used are substantially as described in the previous paper. Normal Bouin's fixative was used in most cases (picric acid, sat. aqueous sol., 75 parts; commercial formalin, 25 parts; glacial acetic acid, 5 parts), as it gives freedom from 'clumping' of chromosomes, at the expense of slight distortion of finer cytoplasmatic details. The fixative recommended by Karl Sax (1), a modified Bouin's solution developed by Allen (2) and containing chromic acid and urea, was tried, but found

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to contain incompatible substances giving rise to violent effervescence and a muddy precipitate on mixing. The chromosomes were counted with objectives of high resolution both directly and with an Abbe drawing apparatus. In most cases the numbers were checked by several independent observers (S. C. H., G. G. C., D. A., and H. G.). Drawings (Figs. 1–11) were made with Zeiss 2 mm. homo. imm. objective, Zeiss K. 18 ocular (new series), and Zeiss large Abbe drawing apparatus, at a magnification of 2,460, reduced in printing to 1,500. Heidenhain's haematoxylin was used in every case. It has not been found practicable to identify the individual chromosomes, which are too minute for the purpose; nor can too much reliance be based on the actual size of the chromosomes, owing to a well-known physiological error introduced in the drawing of small dark bodies on a background of variable intensity. The material was grown from seeds derived from the several sources mentioned. It is noteworthy that whilst no difficulty has been found in getting plants of Sea Island, Upland, and Indian cottons to flower in the greenhouse at all seasons of the year (flowers are self-pollinated and all seed produced is viable), no such success has been obtained with Trinidad Native (tree types) and *Gossypium brasiliense*, while Egyptian cottons are persuaded with difficulty to produce buds, and no cytological material has been obtainable which shows haploid plates.

TYPES EXAMINED.

1. *Sea Island Cottons.*

Gossypium barbadense, var. *maritima*, Watt. From seed of Dr. Harland's pedigree types, brought from St. Vincent.

V. 135.
V. 74.
D. 134.
H. 23–5–21.
B. D. 9–6
U. S. 2.
G. X. 12–2–9–19.
A. E. 17–5–6.
A. R. 3–19.
A. N. 28–19, 13–56.
A. K. 41–18.

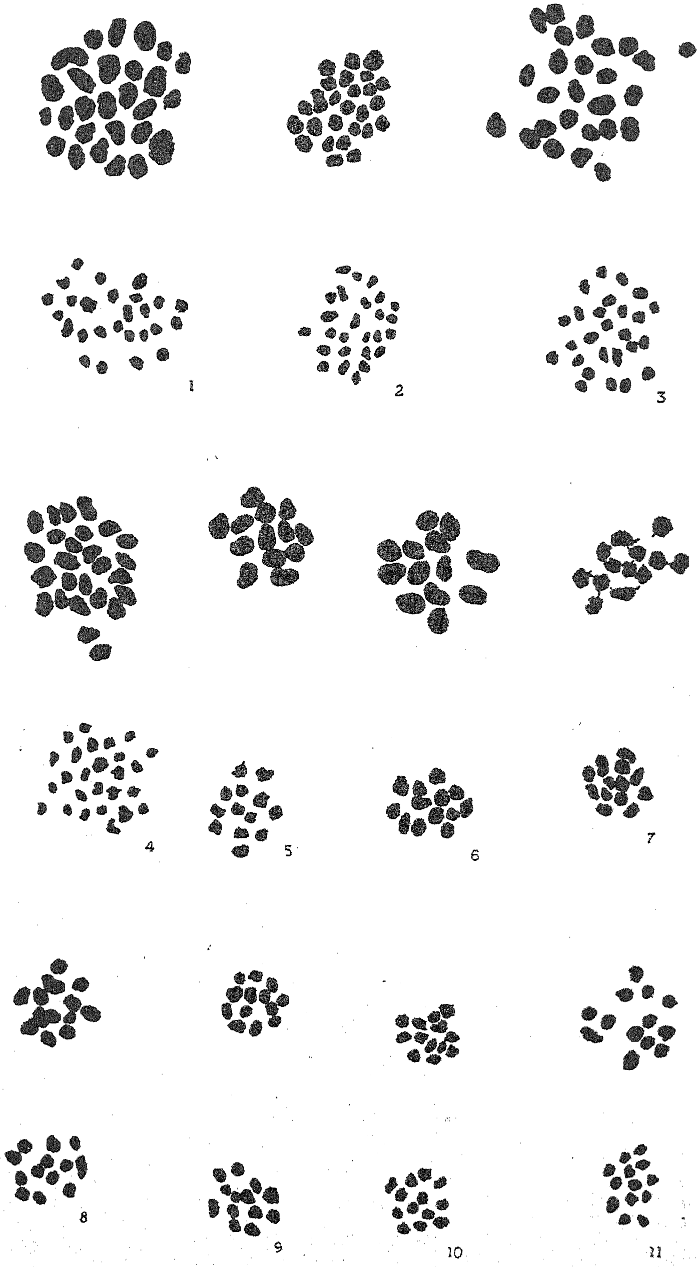
These are the plants from which the material for the previous paper was taken, and agree in having a chromosome number of 26. Two chromosomes are noticeably larger than the remainder.

2. *American Cottons.*

These are grouped by Watt (3) as falling for the most part under *G. hirsutum*, Linn., and *G. mexicanum*, Tod.

Acala. Seed from U.S. Dept. of Agriculture. A large-bolled Upland variety of *G. mexicanum* type 26

Commercial I. Flowered in greenhouse of Botanical Dept., University of Manchester. A commercial cotton of unknown ancestry; small boll, hirsute 26



FIGS. 1–11. Chromosomes of various types of Cotton. 1. Acala. 2. Indian American, 289 F. 3. American Commercial I. 4. American Commercial II. 5. Chinese naked seeded. 6. *G. cernuum* × *rudicum*. 7. *G. arboreum* × *neglectum*. 8. *G. roseum*. 9. *G. neglectum*. 10. *G. arboreum*. 11. *G. sanguineum*. × 1,500

Commercial II. Seed from a plant given by Mr. W. Greenwood, M.P.; a large-bolled, semi-hirsute type, near *G. hirsutum*	26
Indian American, 289 F. Seed of this and the following variety was sent from Cawnpore by Dr. Martin Leake; a small leaf, medium-sized boll, hirsute	26
Indian American, 285 F. A similar plant to 289 F. No details of their ancestry at present available	26

3. *Egyptian Cottons.*

These appear more closely related to *G. barbadense* than to *G. peruvianum*, under which they are placed by Watt.

Mit Afifi. Commercial. Seed given by Dr. W. L. Balls; flowered with difficulty, but no haploid material available. Chromosomes counted in root tip. Diploid number	*circa* 52
Giza I. This and the following are two distinct types, from seed sent by Cotton Research Board, Egypt	26
Giza II. Could only be counted in root tip. Diploid number	*circa* 52
Pima. An American-Egyptian of mixed parentage (see Kearney (4)); probably including commercial Upland and 'Hindi Weed'. No perfect plates	*circa* 26

4. *Indian and Chinese Cottons.*

Seed in every case sent from Cawnpore by Dr. Martin Leake. Chromosome number 13, with one chromosome larger than others.

G. sanguineum, 27, Tod; placed by Watt as *G. arboreum*, Linn., var. *sanguinea*, Watt	13
G. sanguineum, 124	13
G. roseum, Tod = *G. arboreum*, Linn., var. *rosea*, Watt	13
G. arboreum, Linn.	13
G. neglectum, Tod = *G. arboreum*, Linn., var. *neglecta*, Watt	13
G. arboreum × *neglectum*, Leake	13
G. cernuum, Tod = *G. arboreum*, Linn., var. *assamica*, Watt	13
G. rudicum	13
G. cernuum × *rudicum*, Leake	13
G. Mollisoni	13
Chinese naked seeded. Possibly a form of *G. Nanking*, Meyen?	13

5. *Other Species.*

Columbian Native. From seed collected in interior of Colombo by Mr. R. Mordecai. Possibly either *G. mustelinum*, Miers, or *G. peruvianum*, Cav., but not yet grown; from root tip. Diploid number	*circa* 52

The above list is necessarily incomplete, and it is hoped that workers in other parts of the world will be able to record further numbers of this interesting genus in the near future. The types particularly needing elucidation still are the Mediterranean *G. herbaceum*, Linn., and the many other varieties which have been placed under this name; 'Hindi Weed' and the tree cottons of the Sudan; *G. peruvianum*, *brasiliense*, *mexicanum*, *vitifolium*, and many other parents of commercial strains; while the almost hairless aboriginal types, such as *G. Sturtii* or *Sturtia* of Australia and *G. Stocksii* of India, are of great botanical interest. The relations of other genera of the Hibisceae are still questionable, especially the nearly related *Thurberia*, *Fugosia*, and *Thespesia*; whilst there are singularly few numbers known in the whole order Malvaceae, where there is reason to believe that cytological peculiarities abound.

The abrupt separation of the genus *Gossypium* into two groups in which the chromosome number of the one is twice that of the other is in itself a matter of great interest. It is known that interspecific hybrids can be made with comparative ease in either of the groups, but, as far as can be ascertained, no plant-breeder has yet been successful in crossing an American or Egyptian cotton with a true Indian cotton (it has been stated that this cross has been made in Russia, but no reference can be found to it in the available literature, and it is probable that the cross has now been lost). At the same time, it may be observed that the plants of the 26 group are, on the whole, much larger than those in the 13 group; the stems are taller, the leaves, flowers, and bolls are larger, and the lint is longer. It is a matter of speculation whether this is a case of 'gigantism' due to the double chromosome number, though comparable phenomena have arisen in the mutants of *Oenothera* and other species. The fact of the difference may perhaps open a further possibility for the plant-breeder who wishes to make the cross between Indian and American cottons; if it can be assumed that there is any hope of finding a diploid mutant of an Indian cotton (which would probably reveal itself as suitable for cytological examination by its abnormal size) it might reasonably be expected to prove a fertile parent for the experiment.

Until further chromosome numbers are available, it is inadvisable to draw any conclusions as regards the systematy of the genus, which is still in a far from satisfactory state, in spite of the work of Todaro (5), Parlatore (6), and Watt; the question of the identity of *G. hirsutum*, which is described by the latter as common to Europe, Asia, Africa, and America, may be cited as an example. At the same time it is possible that the new evidence obtainable from the chromosome numbers will settle the debate as to the origin of cotton in America. The earlier explorers all stated positively that they found cotton growing and in use (7). Wiener (8), however, has recently made the startling contention that what they saw was not *Gossypium*, but *Bombax Ceiba*, and that cotton was introduced from Europe by Columbus and his followers, or from Africa by the negro overseers of the early plantations—notwithstanding the evidence of the Peruvian tombs.

SUMMARY.

The chromosome numbers have been counted in some thirty-two varieties of cotton, including American, Sea Island, Egyptian, Indian, and Chinese types, and American grown in India. These numbers fall into two groups of 26 and 13 chromosomes, the former comprising the cottons of the New World and Egypt, and the latter those of Asia. This may, perhaps, explain the impossibility of crossing American or Egyptian cottons with Indian types, and suggests a possible solution of the difficulty with the help of cytology.

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2. ALLEN, E.: Studies on Cell Division in the Albino Rat. Anat. Record, 1916, x, 565–84.
3. WATT, SIR G.: The Wild and Cultivated Cottons of the World. London, 1907.
4. KEARNEY, T. H.: Variations in Pima Cotton. Journ. Agric. Res., 1921, xxi, 227–42.
5. TODARO, A.: Osservaz. su tal. sp. di cot.; Relaz. cult. dei cot. 1877.
6. PARLATORE, L.: Le Specie dei cotoni. 1866.
7. DENHAM, H. J.: Gossypium in pre-Linnaean Literature. Oxford Bot. Mem., No. 11, 1919.
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The Problem of identifying the Wood of Cretaceous and Later Dicotyledons: Paraphyllanthoxylon arizonense.

BY

I. W. BAILEY

Bussey Institution for Research in Applied Biology.

With Plate XV.

IT is a striking and well-known fact that the Angiosperms, from the time when they first appear abundantly in the Cretaceous, are highly differentiated and are in general remarkably similar to their descendants in extant floras. The problem of identifying their remains largely resolves itself, therefore, into a study of living plants which provide a fundamental basis for comparison with and determination of fossil forms.

The available palaeontological record of the Angiosperms consists chiefly of impressions of leaves and fruits, and of fossilized fragments of stems and roots; impressions of flowers are of less frequent occurrence. In view of this fact, the question naturally arises as to whether most of the higher plants can be distinguished from one another by characteristic differences in the form and structure of their vegetative organs. Certain systematic botanists are inclined to argue that the flower is inherently more conservative than the leaf or stem, and to cite the occurrence of similar structural characters in widely separated orders and families as evidence that vegetative organs frequently do not afford reliable clues to genetic relationships. Palaeobotanists, while admitting that there is more or less convergence in vegetative organs, maintain that there are differences in the form and structure of these organs which are sufficiently constant to warrant their use as diagnostic criteria. In recent years, a number of anatomists have asserted that internal structures, particularly those of the vascular tissues, are in general more conservative than external floral and foliar characters, and afford surer clues to actual genetic relationships, both among living plants and in fossil floras.

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It should be emphasized in this connexion that most arguments concerning the relative conservatism of specific organs and tissues are based, in ultimate analysis, upon phylogenetic speculations rather than upon extensive statistical and experimental investigations. Thus the putative conservatism of the flower rests largely upon the assumption that an arrangement of Angiosperms in florally homogeneous groups affords a fairly accurate picture of a natural classification, and that the variability of vegetative organs within such groups, therefore, denotes morphological instability. Similarly, the supposedly greater conservatism of internal characters is based upon the assumption that certain sequences of increasing structural specialization are indicative of the general trend of phylogeny, and that such evolutionary series reveal the inconstancy of external foliar and floral characters.

Of course, the absence of a general parallelism in the evolution of floral and of vegetative organs, in itself, does not prove the flower to be inherently more conservative than the leaf, stem, or root, or vice versa. In other words, the occurrence of relatively primitive flowers on highly differentiated stems is no more suggestive of the greater conservatism of floral organs than is the presence of primitive vascular structures, in plants which bear highly modified flowers, suggestive of the greater conservatism of the stem. It appears improbable that the evolution and specialization of all organs and tissues proceeds in all cases and at all times at similar rates and in parallel directions. In fact, there is much evidence, both among plants and animals, that any given organ or tissue may at times remain unchanged when other organs and tissues are becoming profoundly modified. Therefore, if a truly natural classification of Angiosperms is to be formulated, it should be based upon a study of all organs and tissues rather than upon a putatively conservative one. Little would be gained by substituting a classification founded solely upon vascular structures for one based largely upon floral morphology.

As Vavilov[1] has recently pointed out, homologous series in variation tend to occur in floral, as well as in vegetative, organs; not only in closely related Linneons, but also in distantly related families and orders. The fact that certain convergences in vegetative organs and tissues tend to be more or less closely correlated with climatic and edaphic influences does not, however, necessarily prove the leaf or stem to be inherently more plastic or variable than the flower. Such correlations may be interpreted merely as indicating that variations or mutations in foliar and cauline structures are subject to more direct or readily discernible climatic and edaphic selection. Thus, in view of the fact that necessary experimental and statistical data for determining the relative frequences and ranges of germinal and somatic variations in specific organs and tissues are not at present available, controversies concerning the relative conservatism of the flower, leaf, stem, and root appear to be premature and fruitless. It is evident, in addition, that there are no cogent, *a priori* arguments for assuming that Mesozoic and Cainozoic Angiosperms cannot be identified by the form and structure of their vegetative organs ; the only reliable method of proving that they cannot is the laborious one of demonstrating that identical combinations of vegetative characters do occur in different genera, families, and orders, and that such occurrences are so numerous as to invalidate a significant proportion of palaeobotanical determinations.

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[1] Vavilov, N. I.: The Law of Homologous Series in Variation. Journ. Genetics, xii. 47–89, 1922.

A critical study of fossil floras shows that in many cases impressions of leaves which originally were referred to certain genera, families, and orders subsequently are transferred to remotely related ones. Such discrepancies may, of course, be due largely to errors of judgement or to unfamiliarity with the general range of variability of the leaf in the Angiosperms as a whole, rather than to the occurrence of identical foliar complexes in widely separated groups. In fact, the literature of systematic botany is replete with analogous discrepancies, which merely demonstrate that the classification of the higher plants is subject to constant revision and correction. That certain of the earlier palaeobotanists placed too much emphasis upon superficial similarities, were too sanguine in referring leaf impressions to genera in local floras with which they happened to be familiar, and did not devote sufficient attention to foliar characters in groups of Angiosperms which are not represented in such floras, is indicated by the detailed and painstaking investigations of Berry.[1] He holds that, although superficially similar types of leaves, e. g. simple, compound, pinnate, palmate, entire, lobed, serrate, &c., occur in different families and orders, they can be distinguished from one another by very characteristic details of their form and venation. It may be objected, in view of the large numbers of Angiosperms involved, that still more extensive and detailed investigations may prove such diagnostic criteria to be inconstant and unreliable. However, the fact that systematic botanists are able in most cases to determine flowerless specimens of plants from floras with which they are *thoroughly* familiar, suggests that eventually it may be possible to construct keys for distinguishing the foliage of at least a large proportion of the Angiosperms.

In the case of the Dicotyledons, the more conspicuous types of leaf form and venation are not closely correlated with specific types of floral organization. This is due in all probability to the absence of a general parallelism in the evolutionary specializations of the flower and leaf. As indicated in the preceding paragraph, the occurrence of superficially similar types of foliar organs in different genera, families, and orders makes the task of constructing keys for differentiating the leaves of many of the larger groups of

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[1] Berry, E. W. : The Lower Eocene Floras of South-eastern North America. U.S. Geol. Survey, Prof. Paper No. 91. Washington, 1916.

Dicotyledons—as at present constituted by systematists—an extremely tedious and difficult one. It is of interest to determine whether the problem of identifying fossilized dicotyledonous woods is essentially similar to that of determining leaf impressions.

During the last sixteen years, the writer has devoted considerable attention to an investigation of the vascular tissues of the vegetative organs of the higher plants, particularly of the secondary xylem or wood of Gymnosperms and of arborescent and fruticose Dicotyledons. In so doing, he has had occasion to obtain extensive collections of anatomical specimens from various phytogeographical regions of both hemispheres, and from all of the principal orders and most of the larger families of Dicotyledons. Detailed microscopic study of this material, coupled with a critical analysis of available literature in the fields of botany, palaeobotany, and wood technology, reveals the fact that salient lines of structural specialization are very clearly defined in the xylem of Dicotyledons, and that remarkably complete series of transitional stages are preserved in living representatives of the group. Although these evolutionary series do not provide reliable evidence for asserting that vascular structures are inherently more conservative than external foliar and floral characters or vice versa, they afford significant clues concerning unavoidable difficulties which are to be encountered in identifying dicotyledonous woods.

In the older groups of vascular plants, i. e. Calamariales, Sphenophyllales, Lepidophytineae, Cycadofilices, and Gymnospermae, with the exception of the bizarre and structurally highly specialized Gnetales, the secondary xylem was composed of tracheary elements of a single generalized type, the so-called tracheides. This primitive type of wood has persisted in *Trochodendron*, *Tetracentron*, *Drimys*, and *Zygogynum*,[1] but has been modified in the remaining living representatives of the Dicotyledons; certain vertical series of tracheides having differentiated into vessels by the formation of perforations in their adjacent membranes. The comparative anatomy of the various orders and families of Dicotyledons indicates very clearly that the evolution and specialization of vessels is accompanied by other anatomical changes in the xylem, and, in general, is paralleled by concomitant modifications in the structure and activities of the cambium or lateral meristem.

In the structurally less specialized types of Dicotyledons, the wood resembles that of *Trochodendron*, *Tetracentron*, *Drimys*, and *Zygogynum*, except for the presence of numerous perforations in the radial walls of diffusely scattered vertical series of enlarged tracheides. The ground mass of fibrous tissue is composed of tracheides with conspicuously bordered pits;

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[1] Bailey, I. W., and Thompson, W. P.: Additional Notes upon the Angiosperms, *Tetracentron*, *Trochodendron*, and *Drimys*, in which Vessels are absent from the Wood. Ann. Bot., xxxii. 503–12. 1918.

the wood parenchyma is diffuse, i.e. not aggregated in large masses; and such anomalous structures as interxylary phloem, &c., are absent. The fusiform initials of the cambium overlap, and are relatively large and much elongated. Furthermore, the progressive increase in the circumference of the lateral meristem is due primarily to pseudo-transverse, anticlinal divisions, followed by longitudinal sliding growth, of these cells; a type of cambial activity which is characteristic of the more primitive vascular plants.

With increasing specialization, the segments of the vessels tend to shorten,[1] to become relatively wider, blunter, and less angular in cross-section, and thus to lose their resemblance to tracheides. The scalariform perforations in their lateral walls tend to coalesce into large oval or circular openings which ultimately become terminal. At the same time, the cells in the surrounding ground mass of fibrous tissue also tend to become less tracheide-like by a gradual loss of the bordering areas about their pits, as well as by concomitant changes in the dimensions of the elements themselves. As the segments of the vessels shorten, the fusiform initials of the cambium become smaller and elongate less and less between successive anticlinal divisions.[2] This culminates, particularly in various tropical Dicotyledons, in the elimination of longitudinal sliding growth and in the production of a fundamentally different type of cambial activity,[3] in which the anticlinal divisions of the fusiform initials are radio-longitudinal. Such cambia are characterized by having their cells arranged in parallel horizontal series. Therefore, their centripetally formed derivatives tend to be stratified or 'storied', except where the seriation is disturbed by excessive elongation of fibre-tracheides during their differentiation. Frequently, the specialization of the tracheary tissue and cambium is more or less closely paralleled by concomitant changes in the xylem parenchyma and rays.

These salient and more or less closely co-ordinated lines of structural specialization indicate very clearly that the differentiation of the xylem does not in general closely parallel that of the flower. For example, primitive types of vascular structures are retained in certain representatives of the Metachlamydeous orders Ericales, Ebenales, and Rubiales, as well as of the Archichlamydeous orders Garryales, Myricales, Ranales, Rosales, Geraniales, Parietales, Myrtiflorae, and Umbelliflorae. Conversely, very highly specialized ones occur throughout the Archichlamydeae, as well as in the Metachlamydeae. The fact that similar types of vascular structures are found in plants which are placed by systematic botanists in widely separated genera, families, and orders makes the task of constructing keys for distinguishing the woods of many of the larger groups of Dicotyledons an exceedingly difficult one. Such comprehensive orders as the Ranales, Rosales, Geraniales, Sapindales, Parietales, Myrtiflorae, Tubiflorae, &c., exhibit so many different stages in the specialization of their vascular tissues that it is doubtful whether there are any diagnostic characters which are constant in each group as a whole. The same thing is true of many of the larger families. It should not be inferred from this, however, that species or genera having identical *combinations* of structural characters necessarily occur in remotely related families or orders, but rather that the general range of variability of the more salient characters tends to be similar in widely separated groups. Nor should it be assumed that similar stages of specialization of the vascular tissues are necessarily indicative of close genetic relationship.

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[1] Bailey, I. W., and Tupper, W. W.: Size Variation in Tracheary Cells. Proc. Amer. Acad. Arts and Sciences, liv. 149–204. 1918.

[2] Bailey, I. W.: The Cambium and its Derivative Tissues. No. II. Size Variations of Cambial Initials in Gymnosperms and Angiosperms. Amer. Journ. Bot., vii. 355–67. 1920.

[3] Bailey, I. W.: The Cambium and its Derivative Tissues. No. IV. The Increase in Girth of the Cambium. Amer. Journ. Bot., x. 499–509. 1923.

The very highly specialized 'ring-porous' type of structure is a characteristic feature of the wood of various species of *Carya* (Juglandaceae), *Quercus* (Fagaceae), *Celtis* (Ulmaceae), *Toxylon* (Moraceae), *Sassafras* (Lauraceae), *Robinia* (Leguminosae), *Phellodendron* (Rutaceae), *Ailanthus* (Simarubaceae), *Cedrela* (Meliaceae), *Rhus* (Anacardiaceae), *Fraxinus* (Oleaceae), *Tectona* (Verbenaceae), *Paulownia* (Scrophulariaceae), *Catalpa* (Bignoniaceae), &c. To argue that all of these plants are closely related leads to a *reductio ad absurdum*. The typical ring-porous condition arises apparently when plants, which previously have undergone characteristic structural modifications in certain tropical or subtropical environments, are subjected to cold winters or to alternating very dry and wet seasons. In other words, it is closely associated with the acquisition of a pronounced resting period and commonly of a deciduous habit. It is significant that certain species of a widely dispersed genus may be ring porous and others diffuse porous, depending upon their phytogeographical distribution. Although the salient structural features of ring-porous timbers are basically similar, the woods of the various genera enumerated above may be distinguished from one another by characteristic minor differences in the form, structure, and arrangement of their cells, i. e. parallel lines of specialization or modification do not, in this particular case at least, produce identical *combinations* of anatomical characters.

Thus, the problem of identifying fossilized dicotyledonous woods appears to be basically similar to that of determining leaf impressions. In each case, it is essential for the palaeobotanist to become thoroughly familiar with the variability of putative diagnostic characters in all living groups of Dicotyledons and in material from various phytogeographical regions, and to determine whether identical complexes of characters occur in different genera, families, and orders. In the case of the leaf, the extensive collections of successive generations of systematic botanists in the larger herbaria of Europe and of the United States provide abundant basic data for the investigator who wishes to study the foliar characters of the various orders and families of Dicotyledons, or to make comparisons with leaf impressions. Unfortunately, owing to the fact that the structure of the stem and root is not utilized extensively in the classification of Dicotyledons, no comparable collections of these organs are available for systematic investigation, or for reference in the identification of fossil woods. Thus, in attempting to determine the affinities of dicotyledonous woods, the palaeobotanist has been forced to rely mainly upon descriptions and figures which are widely scattered through the literature.

Of course, the publications of morphologists, anatomists, physiologists, and other botanical investigators contain numerous references to the microscopic structure of the vegetative organs of many Dicotyledons. The laborious task of compiling and codifying this information, and of preparing descriptions of the anatomical characteristics of the principal families, was undertaken by Solereder. Although the English edition[1] of his 'Systematische Anatomie der Dicotyledonen' contains an extremely useful classified bibliography of literature, published prior to 1908, and many valuable data concerning the structural peculiarities of various genera, it does not provide reliable criteria for the identification of the wood of either living or fossilized Dicotyledons. In fact, no attempt is made to do more than very briefly outline certain of the more salient features of the xylem in each family. Frequently, the descriptions are based upon inadequate material, i. e. a very limited number of species or genera, or the examination of herbarium specimens whose xylem structurally may be quite unlike that of old robust stems. Therefore, it is not possible to determine the limits of variability of different diagnostic characters in most families. Furthermore, it is evident from the recorded data that similar structures do occur in widely separated groups.

With increasing utilization of new domestic woods and the importation of numerous little-known exotic ones, there arose in the larger timber markets of the world a demand for detailed information concerning the structure and properties of the economic woods of temperate and tropical forests, and for keys for their identification. This has stimulated numerous investigations by botanists, engineers, foresters, and so-called wood technologists. The results of these investigations are of varying degrees of utility to palaeobotanists. Where the descriptions and keys are based upon such macroscopic characters as weight, colour, odour, hardness, elasticity, &c., which are evanescent in fossilized wood, they are of relatively slight value.

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[1] Solereder, H.: Systematic Anatomy of the Dicotyledons. Translated by Boodle and Fritsch. Clarendon Press, Oxford. 1908.

On the contrary, where they are based upon detailed microscopic investigations of authentic material, and are accompanied by numerous photomicrographs or drawings, they may be of considerable significance.

It should be fully recognized and freely admitted, in this connexion, that most determinations of petrified dicotyledonous woods are purely tentative and necessarily subject to extensive revision and correction. That such must indeed be the case is indicated, not only by the general considerations outlined on preceding pages, but more specifically by a critical analysis of keys for distinguishing the principal timbers of commerce. The reliability of such keys varies inversely with the size and complexity of the flora studied. Owing to the limited number of arborescent Dicotyledons in Europe and temperate North America, it is possible to differentiate the woods of the various genera represented in the floras of these regions with a very considerable degree of accuracy. It is also possible to distinguish the woods of most genera in restricted subtropical and tropical floras, although the task is a more difficult one, since in these regions arborescent and fruticose species constitute a much larger proportion of the dicotyledonous flora. A detailed study of available keys indicates very clearly, however, that they cannot be combined at present into a master key to the economic timbers of the world, since many of the diagnostic characters, which are generically stable in one flora or region, prove to be unreliable in others. This appears to be due in part to the fact that widely dispersed genera tend to vary (germinally) in different regions and floras, in part to the occurrence of convergences (germinal) in remotely related genera, and in part to purely somatic fluctuations.

As an illustration of difficulties encountered in studying the affinities of dicotyledonous fossils, the writer may cite the case of some fragments of silicified wood from the Colorado group of Arizona, sections of which were recently sent to him for determination by Dr. F. H. Knowlton. The specimens were collected by Mr. C. P. Ross,[1] of the United States Geological Survey, at the 'North side of headwater basin of Deer Creek, Pinal County, Arizona, about thirteen miles SW. of San Carlos'. The anatomical characters are identical in the various fragments, which appear to have been derived from a single large silicified stem. The more salient structural features are the following:

Paraphyllanthoxylon arizonense, gen. et sp. nov.

Wood parenchyma: large-celled, thin-walled, scanty, mostly paratracheal.

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[1] Mr. Ross reports that fossil wood was fairly abundant. He says, 'All the specimens observed are broken pieces, mostly square ended, from a few inches to about three feet in length. Some of the trunks are more than a foot in diameter. Some are embedded in the rock, others loose on the surface. The probability is that all the fossil wood observed is driftwood that was buried in the rocks and later silicified by circulating solutions which removed the woody matter and deposited silica in its place. Upright stumps in the position of growth are reported to have been found, but were not observed during the present investigation.'

Vessels: large, diffuse, 8–14 per sq. mm., solitary or grouped in radial clusters of 2–4, oval in cross-section or tangentially flattened by mutual pressure; tangential walls between adjacent vessels thick, heavily pitted, pits crowded, hexagonal; vessel segments elongated, with oblique end walls, perforations large, simple, circular or oval. *Tyloses*: abundant. *Fibre-tracheides*: large, thick-walled, septate, pits slit-like, borders vestigial or absent. *Rays*: numerous, large-celled, 1–7 cells wide, 3–80 cells high, frequently as wide as or wider than the intervening radii of tracheary tissue; cells extremely variable in size, shape, and orientation, containing a black, globular substance; marginal cells 1–5, conspicuous, vertically elongated; pits between ray cells and vessels large, transversely elongated. *Growth rings*: feebly differentiated. Tissue elements not stratified or 'storied'.

There is no single, salient structural feature in this fossilized wood which justified its reference to any particular group of Dicotyledons. The form, structure, and distribution of the wood parenchyma, of the vessels, of the fibre-tracheides, and of the rays are of types which occur in remotely related orders and families. The only method of determining its affinities is the laborious one of searching for similar combinations of anatomical characters in extant Dicotyledons. A detailed study of all available data reveals the fact that similar structural complexes occur in certain representatives of the section Phyllanthoideae of the Euphorbiaceae, e. g. species of *Bridelia* and of *Phyllanthus*. Not only do identical combinations of salient characters exist in these plants, but the minor structural details are remarkably similar. Even the rays in the heartwood contain a dark-coloured, more or less globular substance. Although the writer has not succeeded in finding this specific type of xylem in any other family of Dicotyledons, sufficient data are not at present available for hazarding the statement that it is actually confined to the Euphorbiaceae. *A priori*, it appears unlikely that convergences in remotely related orders or families would produce identical combinations of so many different anatomical variables. It should be emphasized in this connexion, however, that there is a very considerable range of structural variability in many of the larger orders and families of Dicotyledons, and that there is considerable parallelism in the principal lines of specialization of the various tissue elements. Therefore, it must be admitted that there is an unavoidable element of uncertainty even in referring the silicified fragments to the Euphorbiaceae.

If they are tentatively to be included in this family, how should they be designated? A customary procedure in describing a Cretaceous or Tertiary petrifaction is to search for some extant wood having a similar type of structure and then to refer the fossil to the genus in which this wood occurs. In so doing various suffixes, e.g. *oxylon*, may or may not be attached to the generic name. Where the resemblance is somewhat tenuous, the prefix *para* is often used. If the fossil happens to be entirely unlike any of the woods with which the investigator is familiar, a new genus is created for its reception. In view of the limited number of extant woods available for comparative purposes, and of the palaeobotanist's unfamiliarity with the ranges of variability of anatomical characters—not only in different species, genera, families, and orders, but also in different portions of a single individual and in individuals grown under different environmental conditions—this procedure of referring certain petrifactions to extant genera and others to extinct genera is somewhat deceptive. It gives the impression of accurate and reliable diagnosis, which frequently has led to erroneous botanical and geological inferences. Thus, on the one hand, the creation of new genera suggests that strikingly different types of anatomical structures existed in the Cretaceous and Tertiary, and, on the other hand, the inclusion of fossilized woods in extant genera implies that these genera actually were represented in the floras of specific geological horizons. As a matter of fact, there is just as great an element of uncertainty in excluding the Lower Greensand *Aptiana radiata* from the genus *Vaccinium*, certain tropical or subtropical montane species of which exhibit similar combinations of anatomical characters, as there is in including various Cretaceous and Tertiary petrifactions in *Lauroxylon*, many of whose salient features are simulated in remotely related genera.

To formulate a less ambiguous nomenclature, i. e. one at once devoid of implications of genetic relationships and clearly and concisely descriptive of complex combinations of anatomical structures, is unfortunately impracticable. A jargon of new binomials cannot be forced upon students of palaeobotanical problems, and would, until generally accepted, lead to much confusion. Of course, fossils may be designated merely by referring to the collector's name and number, as is sometimes done in discussing undetermined herbarium specimens, but there are serious practical objections to the general adoption of such a procedure. It is desirable that the designation of a fossil, particularly in check lists and catalogues, should afford some clue concerning its structural peculiarities and its putative genetic affinities. Therefore, inasmuch as the structure of even the earliest Cretaceous petrifactions of undoubted Angiosperms is closely simulated in living plants, and in view of the fact that radical changes in well-established scientific procedure should if possible be avoided, it seems advisable to continue to refer fossilized dicotyledonous woods to extant genera, but in most cases to use the prefix *para* or some similar term to emphasize the tentative character of the determinations. Accumulations of such form-genera will be attacked by future 'splitters' and 'lumpers', and, as more reliable information concerning the vascular tissues of the Dicotyledons as a whole becomes available, will be assigned to more specific genetic relationships. Thus, the development of this phase of palaeobotany will parallel that of taxonomy in general, which progresses by a series of constant revisions, and leaves a large by-product of synonymy.

As previously stated, the structure of the Cretaceous wood from Arizona is closely simulated in certain species of *Phyllanthus* and *Bridelia*, and the question arises as to which of these genera it should be referred. *Phyllanthus* is at present widely distributed in the warmer regions of both hemispheres and is considered by Ettingshausen to have been present in the Miocene flora of Bohemia. *Bridelia*, on the contrary, is not represented in extant or fossil floras of the New World. Nor is it present in the Upper Cretaceous and Tertiary horizons of Europe. In view of these facts, it appears advisable to establish the form-genus *Paraphyllanthoxylon* for the reception of dicotyledonous woods having combinations of anatomical characters such as occur in mature stems of *Phyllanthus emblica*, L., and other structurally similar representatives of the Phyllanthoideae. To attempt to include all putative Euphorbiaceous woods in a single form-genus, e.g. *Euphorbioxylon*,[1] would be unfortunate. The Euphorbiaceae structurally are an extremely heterogeneous group. So many different stages in the specialization of the vascular tissues are represented that there appear to be no diagnostic characters which are constant in the family as a whole. Thus, a rational procedure is to establish a series of form-genera for a number of specific combinations of anatomical variables.

If it be admitted that it is difficult to distinguish the woods of many extant Dicotyledons, the question arises, in conclusion, as to whether it is desirable at present to attempt to describe Cretaceous and Cainozoic petrifactions. That such fossils should systematically be collected and carefully preserved seems evident. There are, in addition, a number of valid arguments for not delaying their investigation. As is well known, undescribed specimens tend to deteriorate rapidly through neglect and the loss of necessary collateral data. The description of fossilized material not only tends to facilitate its preservation, but leads to an accumulation of detailed information, i.e. descriptions, figures, photomicrographs, &c., which will eventually form the basis for important generalizations. Furthermore, petrifactions of vascular tissues may provide critical evidence in verifying determinations of leaf-impressions. For example, numerous impressions of foliar organs, from various horizons in Europe and North America, are referred to the genus *Populus*, but Berry is of the opinion that certain of these fossils may ultimately be transferred to genera which are at present confined to Japan and Eastern Asia, i.e. *Trochodendron*, *Tetracentron*, *Euptelea*, *Cercidiphyllum*, &c. The structure of the secondary xylem in the Salicaceae is so entirely unlike that which occurs in any of the Magnoliaceae, Trochodendraceae, and Cercidiphyllaceae that their woods may be differentiated with a very considerable degree of accuracy. An additional argument for not deferring the investigation and description of petrified stems and roots of Dicotyledons arises from the fact that fossilized woods, even of uncertain genetic relationships, frequently afford valuable clues concerning Mesozoic and Cainozoic climates. That more or less accurate records of seasonal variations in temperature and rainfall, as well as of specific biological events, e. g. depredations of insects, &c., are at times preserved in the perennial stems and roots of trees has long been known to foresters and botanists. Douglass [1] and Huntington [2] have recently utilized the stems of old trees in studying climatic cycles or more extended periodicity. The writer's investigations indicate that woods from specific environments within the temperate and torrid zones tend to exhibit striking similarities which obviously are not correlated with close genetic relationships. Thus, although it may not be possible at present to determine the exact affinities of most fossilized stems from particular geological horizons, the anatomical structure of these petrifactions may reveal important clues concerning the general environmental conditions of the Cretaceous and Tertiary.

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[1] It seems best to retain this genus for the reception of fossil woods of the structural type described by Felix from the Tertiary of Columbia.

SUMMARY.

1. The relative conservatism of various organs and tissues of Dicotyledons is discussed, and the conclusion is reached that there are no cogent *a priori* arguments for assuming that external floral and foliar characters are inherently more conservative than internal structural characters or vice versa.

2. Salient lines of structural specialization are very clearly defined in the xylem of Dicotyledons, and remarkably complete series of transitional stages are preserved in living representatives of the group. Although these evolutionary series do not provide reliable evidence for asserting that vascular structures are inherently more conservative than external foliar and floral characters, or vice versa, they afford significant clues concerning unavoidable difficulties which are to be encountered in identifying dicotyledonous woods.

3. There appears to have been no close general parallelism in the evolutionary specializations of the flower, leaf, and stem. Primitive types of vascular structures are retained in various representatives of the Metachlamydeae, and, conversely, very highly specialized ones occur in most orders of the Archichlamydeae.

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[1] Douglass, A. E.: Weather Cycles in the Growth of Big Trees. Monthly Weather Review, June 1909.—Climatic Cycles and Tree Growth. Carnegie Inst., Washington, Publication No. 289. 1919.

[2] Huntington, E.: The Climatic Factor. Carnegie Inst., Washington, Publication No. 192. 1914.

4. The occurrence of similar salient vascular structures in widely separated genera, families, and orders makes the task of constructing keys for distinguishing the woods of many of the larger groups of Dicotyledons—as at present constituted—an exceedingly difficult one. In other words, the problem of identifying fossilized woods largely resolves itself into the laborious task of determining whether identical combinations of structural variables occur in different genera, families, and orders.

5. Inherent difficulties in determining the exact genetic affinities of dicotyledonous woods are illustrated by some silicified fragments from the Cretaceous Colorado group of Arizona.

DESCRIPTION OF PLATE XV.

Illustrating Prof. I. W. Bailey's paper on *Paraphyllanthoxylon arizonense*.

Fig. 1. Transverse section, showing size and distribution of vessels. × 35.

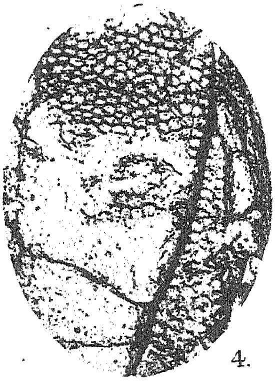
Fig. 2. Tangential longitudinal section, showing size and distribution of rays. Note tyloses in elongated vessel segments. × 35.

Fig. 3. Radial longitudinal section, showing cells of ray. Note large transversely elongated pits and black, granular substance (left). × 160.

Fig. 4. Tangential longitudinal section, showing crowded, hexagonal pits in walls of vessel. × 130.

Fig. 5. Tangential longitudinal section, showing large, thin-walled cells of wood parenchyma chain. × 130.

Fig. 6. Tangential longitudinal section, showing vertically elongated marginal cells of ray. × 130.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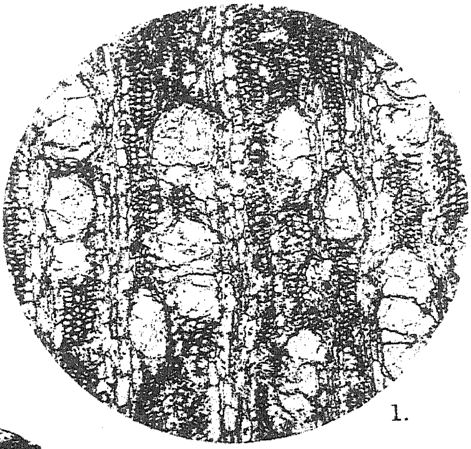
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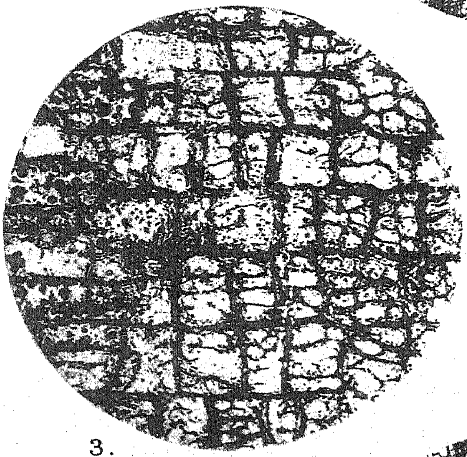
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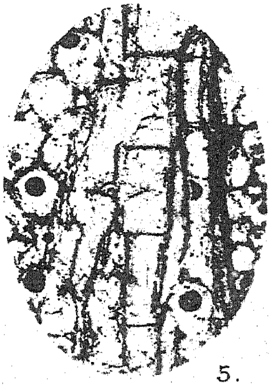
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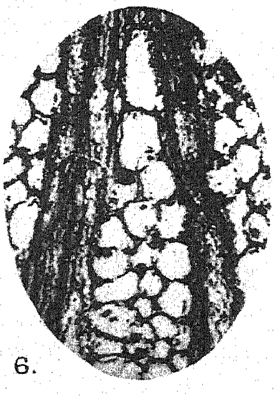
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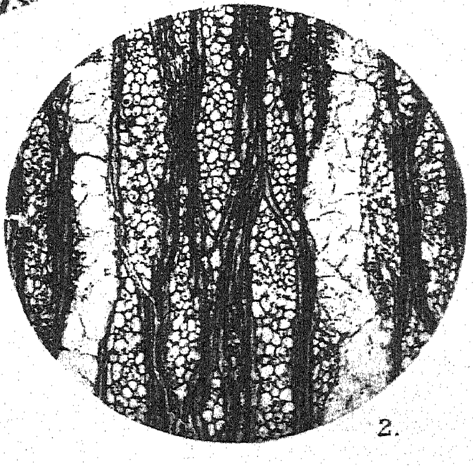
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On the Theory of 'Age and Area'.

BY

S. SCHONLAND.

DR. WILLIS'S 'Age and Area theory' has been before the scientific public for several years. It has been accepted by some botanists who, like Willis himself, have had great experience in plant-geography, while it has been coolly received and even denied by others. Having tested it in many ways, I confess that, like all other theories dealing with evolution since Darwin's time, it leaves me with a feeling of disappointment. Of course its author cannot be held responsible for the extravagant claim made for it in the public press, that it is the most important contribution to evolutionary literature since Darwin's time, and even that it supersedes Darwinism. Darwin's name is in the first place to be associated for all times with the theory that the organic world has developed by evolution. Darwin himself does not claim originality for this view. However, he focused so many facts on it that through him evolution has become the guiding star of the whole scientific world. Secondly, Darwin called attention to the struggle for existence in nature and the consequent survival of the fittest. Comparing this with artificial selection, in which unsuitable varieties are weeded out and suitable varieties accentuated, he called the process which takes place in nature 'Natural Selection', being well aware of the inappropriateness of the term if literally interpreted. Darwin, further knowing the constantly occurring small variations in species, assumed that new species arose by the summing up in one or other direction of these small variations and elimination of those forms which did not vary in the direction that could stand the strain of the struggle for existence. On the question how variations may be produced by the influence of external factors, he brought forward the purely formal theory of pangenesis (which in recent years has to a certain extent been adopted in the hormone theory). I would like to add that Darwinism stands for something else, namely, for honest, patient, and laborious observations, and an almost over-cautious attitude in drawing conclusions from a limited number of facts.

Willis has, in 'Annals of Botany', xxxv, p. 493, briefly summarized his

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ideas, and on reference to this summary and his other published writings it will be seen that, as far as Darwinism is concerned, he comes into conflict with it only on the question of the importance of Natural Selection. In fact, he sometimes seems to wish to supersede it altogether. Starting from the flora of Ceylon, he found 'that local endemics, as a rule, were *not* confined each to one small region or spot characterized by some special local peculiarity in conditions, to suit which they might have been supposed to have been evolved. Not only so, but such spots were frequently to be found with no local species upon them.' 'It was clear that the old ideas of particular adaptation were quite untenable.' Local endemics are according to him, offshoots formed from time to time by widely distributed species. 'The area occupied at any time by any group of species at least ten in number, depends chiefly, so long as conditions remain reasonably constant, upon the ages of the species of that group in that country, but may be enormously modified by the presence of barriers such as seas, rivers, mountains, changes of climate from one region to the next, or other ecological boundaries and the like, also by the action of man and by other causes.' I have no first-hand knowledge of the flora of Ceylon, but I should think that in the first place the struggle for existence in its humid tropical climate must be pretty severe. Now, unfortunately, Willis's statements are not accompanied by any detailed data either of the differences of the species he deals with nor of the exact conditions under which they grow. Instead of statistical returns from various countries a strict investigation of a group of, say, ten species would have been of more value. The conditions in closely contiguous areas may be very diverse though apparently similar; the adaptations to meet them may be associated with different stages in the life-histories of the plant, and may even be expressed by internal characters. No superficial examination can convince us that new species are simply dropped like ripe apples from a tree, though the rôle of the polymorphous or highly variable species in the development of new forms has been recognized long ago, as is shown amongst others by Guppy in the same number of the 'Annals of Botany' (l. c., p. 517). Willis is fighting imaginary foes when he tries to prove that all endemic species are supposed by botanists generally to be on the point of extinction. He asks: 'Why did so many choose mountain tops as a last resort?' Now I imagine that mountain tops, even in Ceylon, exhibit meteorological or even edaphic conditions not generally found in other parts of Ceylon. How does this statement of his tally with his assertion that local endemics in Ceylon were not found, as a rule, in one spot or small region characterized by some special local peculiarity in conditions, to suit which they might have been supposed to have been evolved? European botanists see, perhaps rightly, in some of the alpine plants of their continent remnants of an ancient glacial flora, but this does not apply to all alpine plants in Europe, and certainly not to the alpine plants of

South Africa. The latter must largely be relatively young species, but the point here is that Natural Selection—the survival of the fittest in the struggle for existence—seems to be the only feasible interpretation of the fact of their existence. The large majority of our South African alpine plants are clearly related to others growing at lower altitudes, and any attempt to derive the latter from the former is, in a large majority of cases, futile on the face of it.

My friend Dr. J. W. Bews has in several papers given his blessing (though not unqualified) to Willis's theory. In his paper on 'Some General Principles of Plant Distribution as illustrated by the Flora of South Africa' ('Ann. of Botany', xxxv. 1), he has collected a number of mostly well-known facts to show the relation of numerous localized forms, species, genera, and larger groups, to more widely spread forms, but the large majority of his facts can be interpreted as a vindication of Darwin's theory of Natural Selection.[1] He says on p. 31: 'The whole of our argument is based on the hypothesis that a species in the course of its migrations, when it comes into contact with conditions different from those which produced it, is in many cases at least capable of differentiation or of giving rise to new species suited to the new conditions. In a larger sense, and over a longer period of time, the same applies to larger groups, the genera, tribes, and families.' Nobody will find fault with these conclusions. They support Darwin, and they would also support Willis's statistical methods. Willis, however, goes a step farther and maintains that all endemics must be relatively young. Of course, even Willis cannot close his eyes to the fact that various conditions might modify the action of his 'Age and Area rule'. Some of them, according to him, come into action in almost every single case of any individual species, though upon large numbers and in the long run they cancel out—a perfectly gratuitous assumption, as Bews has pointed out in his paper on 'Plant Distribution in South Africa' ('Ann. of Botany', xxxiv, p. 289). Willis's replies to various criticisms seem to me to be far from satisfactory. In some of his statements I cannot follow him at all, e.g. when he makes a distinction between taxonomic and ecological distribution of plants. I cannot even acknowledge that there is a clear distinction between ecological and taxonomic characters, though the taxonomist, as a rule, only makes use of a limited number of characters. The anatomical characters of *Passerina*, for instance, which Thoday described for two species ('Ann. of Botany', xxxv. 585), are to my mind just as much taxonomic characters as the external characters of the leaf, to which the taxonomist usually only refers in his descriptions. On the other hand, there are taxonomic characters which are not necessarily of any ecological value. Amongst them are, e.g., the meristic characters of a number of species of *Crassula*. I will only refer to one of them. *Cr. multicava*, Lem. (*quadrifida*, Bak.), has individuals with tetramerous and others with pentamerous flowers. They do not differ essentially in conspicuousness. Both produce innumerable seeds, and have also extensive vegetative reproduction. As far as one can see neither of them is favoured or handicapped in the struggle for existence. To this category of taxonomic characters belong also a number of rudimentary organs, such as the rudimentary perianth of many *Cyperaceae*, e.g. the species of *Fuirena*, also the so-called perigynium of *Ficinia*, and possibly of other genera of *Cyperaceae*. However, I have shown (' Introduction to South African *Cyperaceae*', Pretoria, 1922) that these only deserve a subordinate taxonomic value, if any at all, and this applies also to meristic characters of the flowers of the genus *Crassula*.

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[1] Here and there Bews has committed minor errors, e.g. when he associates the local *Podocarpus falcata* with the more widely spread *P. latifolia* and derives the former from it. The two species belong to quite different sections of the genus.

We must now give a little attention to the various causes or conditions that may vary the 'Age and Area rule', but which, according to Willis, cancel out in the long run when sufficient number of species are considered. I will consider these only in connexion with the South African flora, and naturally an exhaustive treatment will not be attempted. Moreover, this point has been dealt with in various papers by Bews, and I will try to avoid as much as possible the evidence brought forward by him. We will take them in the order in which they are enumerated by Willis in 'Annals of Botany', xxxi. 206.

1. *Chance* (*the operation of causes as yet not understood*).—Amongst these causes not well understood I include the action of parasites and herbivorous animals, changes in the chemical composition of the soil (including soil-acidity), micro-organisms in the soil, &c. Too little is known of the operation of parasites (both vegetable and animal) except in the case of cultivated plants, but we know of so-called biological species of Fungi, and we know that these may change their biological characters. On the other hand, we may well assume that just as in the cultivated higher plants, so there are forms in nature which can resist fungal parasites which could destroy other forms. There is here a real struggle for existence in which the fittest only will survive. Such a struggle is also frequently waged with angiospermous parasites and even with climbing plants. A very interesting case which is worth watching for years to come was brought to my notice by an excellent observer, Mr. J. D. Keet, formerly District Forest Officer at Knysna, now Conservator of Forests at Pretoria. He sent me a species of *Cuscuta* (which turned out to be undescribed—its origin is unknown) which attacks various plants near Knysna, but especially the Keurboom (*Virgilia capensis*). The parasite has only been known for a few years, but is getting serious in its damage to the Keurboom. Now in the first place the Keurboom is an isolated genus and species occurring only in South-west Cape Colony. Most of its associates in the forests of the south-west coast region are clearly

of tropical African origin. Has it also been derived from tropical Africa and been restricted to the south-west coast region by parasites? It has the power of springing up readily again when native forests have been cleared by fire or otherwise, and many other arborescent types in the Knysna–Zitzikamma forests can only come up if shaded by it. If we were to assume that the *Cuscuta* would kill out the Keurboom and not even attack other arborescent plants, it is pretty safe to say that these forests would disappear in time. The operations of herbivorous animals have also received too little attention hitherto. It would astonish any European botanist to see the large variety of plants that serve as food in South Africa for grazing domesticated animals, especially in times of drought, and for antelopes (numerous both in species and even in individuals), as well as elephants, buffaloes, zebras. These must have exercised their share in the struggle for existence of our plants even before the advent of man and his flocks of cattle, sheep, and goats. They will have found out the titbits and given the plants with nasty tastes full of tannine a chance, and physiological peculiarities must in many cases at all events have found their expression in external morphological characters. Spinosity may have been accentuated in the same fashion. In dealing with herbivorous animals, we must not forget rodents, which are also represented in South Africa by numerous species and individuals, and lastly insects—the herbivorous beetles, caterpillars, locusts, &c. I have included locusts under the first heading because no accurate studies on their influence on the South African flora are available, and because usually to a large extent their depredations, though frequently very severe, are not regular; e. g. the coast regions are rarely visited by locusts, whereas in former years at all events, before they were artificially checked, they were sometimes for years found in countless numbers in the interior districts. These and other causes may well account for the discontinuous distribution of many plants in South Africa, and for the fact that many endemics may be very old as compared with species of much wider distribution.

2. *Action of man.*—This has been a potent factor in the distribution of South African plants from prehistoric times onwards. The pasturage was fired, especially towards the end of winter, by the natives of the coast districts before white settlers arrived. The object was to give the flocks the benefit of the young shoots in spring, or attract game to these spots. To this day the same practice largely prevails in those parts in which the pasturage will burn. It is a common sight to see large fires extending sometimes over hundreds of square miles in Bechuanaland and right into the Kalahari. One of the finest sights I saw was the grass burning at night on the kopjes near Pretoria when I first visited the capital, and of course I have seen innumerable veld fires elsewhere. The effects of these fires on the vegetation are different in different parts of South Africa, but

they have not been sufficiently studied in connexion with the question under discussion, though their importance cannot be denied, and they must have played havoc with the mechanical rule of distribution which Willis postulates. Forests especially have suffered greatly through fire, but also through felling, and the practice of the Kaffirs of using saplings for the construction of their huts has in parts of South Africa greatly influenced forest growth. However, the greatest changes in the vegetation of South Africa have been brought about during the last 100 years through overstocking and the system of kraaling, owing to which flocks return every night to fixed points, forming beaten tracks which through heavy downpours get washed out and eventually form deep dongas, which lower the water-level and also involve a rapid run off. Thus in innumerable places a much smaller percentage of water that comes down as rain is kept back for the use of plants, aridity increases, and plants with xerophytic adaptations are getting a greater and greater advantage. Since evolution of plants in South Africa has plainly been in many cases towards increasing xerophytism, the 'Age and Area rule', if it is conceded that theoretically it may exist, must have been greatly interfered with in South Africa during comparatively recent times. The ever-increasing flocks of the inhabitants of South Africa have also led to diminution of the number of individuals of certain species of plants. Ostriches especially are responsible for the practical disappearance in certain parts of Stapelias and certain fleshy species of *Euphorbia.* Perhaps Angora goats have even been more destructive. Overstocking is also largely responsible for the enormous spread in pasture lands of weeds, both native and others introduced from other countries by man. The latter are not particularly numerous in species (apart from those on cultivated ground and on roadsides), but some of them have an enormous distribution now and have frequently ousted native plants.

3. *Interposition of barriers.*—In a continental area like South Africa there are especially (1) mountain ranges, (2) valleys, to be considered. These play an important part in South African plant distribution. They mostly act as climatic barriers. Our mountain ranges running chiefly parallel with the coast have on the whole a very good rainfall. In the south-west especially they are favoured by mists and frequent slight showers. Passing over their crests to the side away from the ocean, the rainfall drops suddenly and the mists do not go beyond them. This involves a climatic barrier which in innumerable cases is quite startling to behold. From dense growth with Proteaceae, Restiaceae, Bruniaceae, and other south-west types, one passes almost abruptly into Karroo vegetation. A similar abrupt change is usually seen between grassveld and Karroid scrub in the south-eastern parts, but not between grassveld and Karroid shrublets, to which reference will again be made presently. Our valleys are generally much drier than the adjoining country, and the valleys of the Gauritz River,

the Gamtoos River, the Sundays River, the Great Fish River, and the Kei River form important phyto-geographical barriers. Permanent climatic barriers are also given by the seasonal distribution of rainfall. The South-west Cape Province has essentially winter rains, the remainder of South Africa essentially summer rains, but such permanent climatic boundaries might well fit in with an 'Age and Area rule'. It is different with fluctuating climatic barriers owing to periods of drought and comparatively good rainfall alternating at irregular intervals. This applies to the greater part of South Africa, though not to the same extent to South-west Cape Colony as to the remainder. It is especially striking in its results in the eastern parts of the Karroo (a badly defined geographical term meaning the interior part of the Cape Province, in which the vegetation consists largely of dwarf shrublets). Here a battle royal is waged year after year between grasses and dwarf shrublets (though the struggle is now in many places obscured through the overstocking of many farms). For years not a blade of grass is seen over wide areas. Even right into the south-east portion of the Orange Free State the Karroo shrublets may reign supreme. In times of good rainfall the picture is changed. In April 1921 the greater part of this area, as far west as Beaufort West, looked like typical grassveld. Of course only a limited number of grasses specially adapted to these strange conditions can take part in the struggle, and it is no use drawing up statistical returns for these large areas. The conclusions as to their 'Age and Area' must be wrong. Much less is this justified in comparing the grasses of this area from this point of view with those of the adjoining so-called Kalahari desert (which to a large extent is not a desert at all), where the moisture is somewhat tenaciously retained in the upper layers of the sandy soil.

4. *Geological changes.*—These, of course, go on incessantly, but no violent changes have taken place in South Africa since cretaceous times. Previous changes may perhaps be ignored when dealing with flowering plants. The fact that soils have been derived from different geological formations has in South Africa only minor importance (just like altitude). A few examples may suffice. Karroid scrub formation and Karroid shrublet formation of more or less the same composition in each case are near Uitenhage not much above sea-level on recent deposits, Pleistocene, on a cretaceous formation, and even on Table Mountain sandstone and Bokkeveld beds (carboniferous). Similar plant-formations are found near Grahamstown on Witteberg sandstone (to an altitude of about 2200 ft.), on the Dwyka and Ecca series (both triassic). The country round East London belongs to the Beaufort series (triassic) and has essentially permanent grassveld. On the same series we find the forests of the Amatola Mountains and a large part of the Karroo vegetation farther west. Available moisture is here the limiting factor, and this shows itself, not only directly, but indirectly by its

effect on the soil, determining by leaching largely its chemical composition and no doubt also influencing very largely the development of micro-organisms.

5. *Serious changes of climate.*—There is no evidence to show that since cretaceous times serious changes of climate have taken place in South Africa as a whole, though there must have been numerous local changes, owing to lowering of mountain ranges, deepening of valleys, extermination of forests, &c. It has also been shown (Du Toit in 'S. A. Geographical Journal', December, 1922) that in the Pleistocene there was temporarily an extension of the land towards the south. This must have influenced the climate of a portion of our now very narrow coast districts.

Some of the remaining points have been more or less incidentally dealt with by me. I may be allowed to say that I cannot see on what logical grounds they have been put down by Willis. In most cases I will confine myself to the enumeration of his points.

6. *Natural Selection.*

7. *Local adaptation.*

8. *Dying out of occasional old species.*

9. *Arrival of a species at its climatic limit.*—The importance of this point is frequently exaggerated. We see this plainly in cultivated plants. In the Bathurst division, e.g., apples and pine-apples are grown to perfection almost side by side. The only native palm which reaches the Cape Colony, *Phoenix reclinata*, is distributed in a narrow coast strip as far as the mouth of the Bushman's River. This has often been ascribed to temperature limitations, but the temperature is pretty even along the coast for hundreds of miles westward from this spot, and it can be grown and produces ripe fruit inland even in exposed places, e.g. at Grahamstown, where there is heavy frost in winter. The reason for its limited extent is probably to be sought for in the distribution during the fruiting season of birds, especially parrots, which disperse its seeds. Bews has shown that in Natal tropical plants ascend from a narrow coast strip in the river valleys to localities with more temperate climates with the aid of birds. Thus the distribution of our plants cannot be explained by the study of plants alone. I may here mention in parenthesis that it may frequently depend on pollinating insects, the distribution of which may be governed by different circumstances than those of plants, on insects destroying seeds, &c.

10. *Density of vegetation upon the ground at the time of arrival of a species.*

11. *Presence or absence of mountain chains.*

12. *Relative width of the union between the country of departure and that of arrival.*

It is not likely that a general rule of 'Age and Area' can be drawn up for South Africa. These points, according to Willis, cancel themselves out in the long run, but if they did, this would be quite accidental and the result would not teach us anything.

Endemic species and genera.—The term 'endemic' may be applied to South Africa as a whole, or to one region of it, or to a more limited area only, in which case the term 'local' may be preferable.

Willis states ('Ann. of Botany', xxv, p. 503) that 'there is no difference between endemic genera and species and others that occupy larger areas, except that in general they are younger'. As far as I know, no botanist has ever asserted that all endemic species and genera are relics, but there has, on the other hand, been too great a tendency to deduce relative age by means of 'evolutionary tendencies' and by facts of present-day distribution, and thus construct evolutionary schemes in which many endemic species and genera form the ultimate branches. The statistical tables constructed by Willis in the paper referred to, as well as in others, do not seem convincing. They group together such heterogeneous elements that by their separation and closer analysis quite different results might be obtained. If they prove that the endemic genera on the islands to which he refers are not moribund he will have hurt nobody's feelings. This conclusion might be accepted from Darwinian principles, but Willis's principles do not tell us which of them are relics, and he himself admits that such do occur. In fact there is a postscript to this paper in which he admits that in North Temperate America amongst the endemic genera there are many relics, while there are, according to him, none in the tropics nor in Europe. In the same number of the 'Annals of Botany', Guppy, by the irony of fate, has a paper, 'The Testimony of the Endemic Species of the Canary Islands in favour of the Age and Area Theory of Willis' (l. c., p. 514), in which a return to a pre-Darwinian standpoint is urged, which according to the author is Willis's standpoint, but my poor understanding cannot follow him when he praises Pitart and Prouet's work as being on the lines of Willis's theory, and immediately afterwards states, 'Evidently they hold with Hooker that the single species have arisen as adaptations to the particular conditions of individual islands'. The parent species, of course, must have reached these islands and died out. If this is not pure Darwinism I do not know what to call it. He also states that 'the later evidence indicates that the Canaries and the Macaronesian group generally are by no means alone in this respect' (that they hold relics of an ancient continental flora), 'and that islands have often been sanctuaries for the survivors of continental floras that have passed away'. There is nothing pre-Darwinian or anti-Darwinian in these utterances either. Hooker's views on these endemic species were based on the discovery of plants in the tertiary beds of southern Europe, closely allied to or identical with living Macaronesian species. The past has here been a guide to the present, and where we have, as in the greater part of the world, and certainly in South Africa, to take the present as a guide to the past, we have to be extremely cautious and cannot in many cases come to any conclusion whether an endemic species or genus is a relic and therefore, according to Willis, necessarily moribund (which I do not admit), or whether it is some recent creation.

A few examples may illustrate that great caution is necessary. *Crassula natans*, Thunb. (*Helophytum natans*, E. et Z.), was until recently only known from the southern coast districts of Cape Colony and extends in a robust variety to East Africa, but a few years ago it was also found in Australia, where no near relation of the species occurs.

Cyperus tenellus, L. f., is found in the southern coast districts of Cape Colony as far east as Grahamstown, also in temperate Australia and New Zealand. If either of these species were to die out in one of their remote homes they would be classed as endemics and as young species, whereas they are clearly relics.

Chrysithrix, L., was until lately thought to be endemic in South-west Cape Colony. C. B. Clarke has described an Australian species in 1909.

Schoenoxiphium, Nees, which the latest monographer of *Cyperaceae-Caricoideae*, Pastor Kükenthal, regards as the starting-point of this group, is almost confined to South Africa. It extends slightly to Nyasaland. Now the huge genus *Carex*, though represented in South Africa, is not likely to have originated here. One must, therefore, conclude *Schoenoxiphium* is a relic. The Keurboom (*Virgilia capensis*) is an endemic in the narrow coast strip from Van Stadens westward. The genus *Virgilia* is monotypic. It is allied to *Calpurnia*, which is found in the warmer parts of Africa, and one species in the East Indies, but it stops short in the Uitenhage division just about where *Virgilia* begins. *Virgilia* is also allied to *Bolusanthus*, a genus widely spread in southern tropical Africa. These genera belong to the *Sophoreae*, mostly tropical genera which are generally considered to include types which represent or are near the ancestors of *Papilionaceae*. In any case *Virgilia* appears to be a relic. Amongst the genera which are clear relics in South Africa are *Welwitschia*, *Prionium*, and *Hydnora*. Then there is e.g. the genus *Greyia*, only found on the Drakensbergen and near Kormgha. *Greyia*, though it has distant relatives in South Africa (*Melianthus* and *Bersama*), must be a pretty old genus, and does not fit in with an 'Age and Area rule'. Another and perhaps more striking example is my genus *Pagella* ('Ann. Bolus Herb.' iii. 67), which must also be very old. It was found a couple of years ago at Montagu and Matjesfontein. Though easily overlooked it cannot have an extensive distribution. It is quite isolated amongst the genera of *Crassulaceae*.

A large number of genera radiate from the tropics into South Africa and vice versa (compare my 'Summary of the Distribution of South African Flowering Plants' in 'Trans. Royal Soc. of South Africa', vii. 19). In many cases the relation of 'Age and Area' can be *surmised*. Even in not a few cases there is a general agreement on these points amongst botanists who have given attention to the subject. There was no need to invent for these an 'Age and Area theory', but to speak of a *general* 'Age and Area theory' or of an 'Age and Area rule' is absolutely unjustified. It would be very helpful if it existed, but a theory or rule dealing with 'Age and Area' of plants is of no use unless it helps us to understand present-day distribution and to distinguish plainly the derived from the primitive. If such a rule could be of any value at all, it should be possible to arrange the genera according to distribution, and thus arrive at a natural classification. Everybody will admit that this is an impossibility for South Africa and for any area, small or large, that we may select, except in such a proportion of cases as we may expect if we accept the theory of evolution. A more decided relation between 'Age and Area' will naturally be expected by anybody believing in evolution in closely allied species, but even here we cannot acclaim a theory which leaves us in doubt in most cases, and is clearly useless in many others. If we find, as is actually the case, a *Luzula* or a *Vaccinium* in South Africa, it is reasonable to conclude that they have reached the country after allied species were developed in the north, and that they are derived from them. We conclude that they are comparatively young. *Sanicula europaea* is found in South Africa unaltered, but even this fact does not necessarily mean that it is a younger South African inhabitant than our *Luzula* or *Vaccinium*. There is nothing inherent in a species which compels it to vary. Thus it is clearly unjustified to lump together in statistical returns dealing with 'Age and Area' genera with no near relationships amongst themselves. I shall deal presently with a few cases to show that even amongst allied species application of an 'Age and Area theory' is of no value, but I would first of all call attention to a few cases of discontinuous distribution of species in South Africa which will show that we may well be content to apply the principles laid down and used by Sir Joseph Hooker, Asa Gray, Engler, and other distinguished phytogeographers. The leading ideas with which Engler prefaced his 'Versuch einer Entwicklungsgeschichte der Pflanzenwelt' (1879) have no way been disproved or even amplified by anything Willis or his supporters have published, although they may well here and there require revision on other grounds.

Species with discontinuous distribution in South Africa are found especially in the coastal districts. In some cases they may only appear to be discontinuous owing to our ignorance; e.g. *Costularia humilis*, which was found over 100 years ago near Cape Town, has only recently been found again near Muizenberg, and soon after near Knysna, but it is a plant easily overlooked. This may apply also to *Crassula glomerata*, which occurs near Cape Town, the mouth of the Slang River, and near Humewood. Until recently it was only recorded from South-west Cape Colony. In the present state of our knowledge of plant distribution in South Africa, one can in many cases reasonably expect to see widely separated stations connected by suitable localities at intermediate stations, but there are evident exceptions, that is, of species with widely separated stations with suitable localities between them in which they are undoubtedly absent.

In the Knysna-Zitzikamma forest area we find e.g. *Faurea saligna*, *Ocotea bullata*, *Pygeum africanum*, *Calanthe natalensis*, *Strelitzia augusta*, *Dumasia villosa*, *Anemone capensis*, and a number of other plants which are separated by hundreds of miles from the nearest station farther east, although there are innumerable suitable localities for their existence in between. If they disappeared in the Knysna-Zitzikamma area, where most of them are very local, their area would at once be changed. The few examples I have chosen are not isolated ones. Hundreds of others could have been given.

The crux of Willis's 'Age and Area theory' consists in the assumption that the dispersal of a species and of the new species derived from it must be centrifugal. Theoretically, this may be conceded at the start, but by no means for all times to come.

If a species *A* spreads it gives rise to new species *B*, *C*, *D*, &c. The longer it has been in one place the greater, according to Willis, will be the number of derived species which show themselves as local endemics. He comes to the conclusion ('Ann. of Botany', xxxv. 427)—in the first place in the case of the New Zealand flora—that the number of endemic species would gradually rise to a maximum at or near the point where the genus entered a country. By the massing of endemics he tried to show that he could discover separate invasions. Now, in the first place, there is absolutely no reason to think that an endemic may not spread in a direction just opposite to that from which the parent came, and even occupy in time a greater area if it is better fitted out in the struggle for existence. In South Africa we have a number of genera which are not likely to have originated here, e.g. *Crassula*, *Helichrysum*, *Matricaria*, *Erica*. They have a large number of endemics, many of them local, but none of them can have entered the country at the centres where their endemics are chiefly massed. The genus *Erica* shows this most clearly, and is also more easily dealt with, as it does not penetrate far from the coast into the drier interior parts. A few remarks on the distribution of this genus in South Africa and its relation to that in tropical Africa and Europe, as well as of other *Ericaceae* generally, may, therefore, not be out of place. I may preface them, however, by the remark that Willis's 'Age and Area theory' does not in the slightest degree help us to gain a clearer insight into their distribution. Willis insists that one must take species of the same ecological type for comparison, and at least ten species if one wants to come to satisfactory conclusions, and the genus *Erica* and allied genera are, therefore, admirably adapted to serve as a test. Moreover, all *Ericaceae* are conspicuous plants, collected even by amateurs, and their distribution, though far from thoroughly known, is better known than those of the majority of other South African Natural Orders. I may say that with these difficult plants I have had the advantage of the kind services of the late Dr. Bolus, Mrs. F. Bolus, and Miss Guthrie in the determination of much of my material not quoted in the 'Flora Capensis'. All four subgenera and the large majority of the forty sections into which the genus has been subdivided occur in a preponderating degree west of George in the region of decided winter rains. East of Van Stadens the genus tends to be restricted to the mountain ranges with a more temperate climate than localities of lower altitudes. In considering the table on p. 466, in which the number of species found in the George–Knysna–Zitzikamma area are compared with those near Port Elizabeth, chiefly at Van Stadens, on the Zuurberg near Grahamstown, and on the Drakensbergen, one may well conclude at first sight that the distribution in these areas is a proof of Willis's 'Age and Area theory'. Curves could be drawn which would resemble those given by Willis for New Zealand species of *Ranunculus* ('Ann. of Botany', xxxv. 428, 499), apparently proving that the genus reached South-west Cape Colony first. But the whole theory as applied to this case tumbles to pieces—

1. When we analyse groups of species;
2. When we take into account allied genera which appear to be derived from the genus *Erica*;
3. When we take into account the distribution of the *Ericaceae* generally, and of *Erica* in particular, which seem to prove that *Erica* has arisen in the Northern Hemisphere. There is not a single fact known which indicates southern origin. It must have come to us through the mountains of tropical Africa.

Erica, L.

Distribution of South African species east of George.—The total number of species mentioned in the first column is taken from the 'Flora Capensis', vol. iv. These figures should here and there have been slightly altered owing to new species having been described since its publication. The alterations would, however, not affect the arguments.

The areas mentioned are particularly suited for comparison on Willis's 'Age and Area theory', as they may be compared to islands more and more remote from a central one. The general results would not be materially altered by linking up intermediate stations such as the Amatolas and the mountains near Queenstown, with the Zuurberg on the one hand and the Drakensbergen on the other hand. One species, namely *E. reenensis*, Zahlbr., from the Drakensbergen, could not be accurately placed by me and is not included.

This scheme shows in the first place that the number of sections

	Total No. of species (mainly or entirely W. of George, except 32, 35, 40).	*George–Knysna–Zitzikamma area to Humansdorp.*	*Near Port Elizabeth (chiefly Van Stadens).*	*Zuurberg nr. Grahamstown.*	*Drakensberg and Natal.*
I. Subgenus *Syringodea*	80				
1. *Gigandra*	5	2 (1 at George only)	—	—	—
2. *Didymanthera*	6	1	—	—	—
3. *Pleurocallis*	24	2 (1 at George only)	—	—	—
4. *Evanthe*	35	9	3	1	—
5. *Dasyanthes*	7	3	3	1	2
6. *Chona*	1	—	—	—	—
7. *Bactridium*	2	—	—	—	—
II. Subgenus *Stellanthe*	42				
8. *Euryloma*	15	—	—	—	—
9. *Ceramus*	5	—	—	—	—
10. *Callista*	11	1	—	—	—
11. *Platyspora*	6	4	1	—	—
12. *Myra*	5	—	—	—	—
III. Subgenus *Eu-Erica*	221				
13. *Ephebus*	53	1 (or 2 ?)	1	2	4
14. *Ceramia*	32	4 (3 at George only)	1	—	1
15. *Desmia*	3	—	—	—	—
16. *Gypsocallis*	8	2	1	—	
17. *Pyronium*	14	6	5	3	1
18. *Orophanes*	27	6	—	—	2
19. *Leptodendron*	9	1 (?)	—	—	—
20. *Pachysa*	24	5	—	—	2
21. *Hermes*	15	—	—	—	—
22. *Chlorocodon*	7	2	1	—	3
23. *Arsace*	16	9	2	1	5
24. *Pseuderemia*	9	1 (near George)	—	1 (?)	3
25. *Polydesmia*	4	1	—	—	—
IV. Subgenus *Chlamydanthe*	88				
26. *Chromostegia*	3	—	—	—	—
27. *Oxyloma*	3	—	—	—	—
28. *Eriodesmia*	4	1	—	—	—
29. *Amphodea*	3	—	—	—	—
30. *Geissostegia*	15	4	—	1	—
31. *Elytrostegia*	5	1	1	—	—
32. *Apoecus*	2	—	—	2 (or 1 ?)	1
33. *Lamprotis*	20	4 (2 near George only)	1	—	2
34. *Eurystegia*	10	—	—	—	—
35. *Adelopetalum*	1	1	1		
36. *Trigemma*	22	3 (or 2 ?)	1	—	2
V. Subgenus *Platystoma*	38				
37. *Polycodon*	8	2	2	2	—
38. *Eurystema*	11	2	1	1	—
39. *Melastemon*	10	4	2	—	1
40. *Gamocalamys*	6	4	3	1	1
41. *Cyatholoma*	3	—	—	—	—

decreases from west to east, and also on the whole the number of species. It would lead me too far to reproduce a detailed analysis of comparison of all the species considered by me. I will confine myself to a few selections, which show that Willis's 'Age and Area theory' does not find confirma-in these heaths.

4. Section *Evanthe.—E. curviflora* is widely spread in the Knysna–Zitzikamma area and extends westward to Clanwilliam. It is a very variable species, but there are other variable species in this section with restricted distribution in the south-west, and there is nothing to show that it has more primitive characters than the others.

5. Section *Dasyanthes.—E. cerinthoides* is very variable, but only one distinct variety can be well defined. It is probably the most widely spread species in South Africa. It reaches the habitat of the only other species of this section found east of Port Elizabeth, namely *E. Oatesii*, which is only known from the Drakensbergen. This, however, seems to be a more primitive form. According to Bolus and Guthrie it almost unites the sections *Dasyanthes* and *Ephebus*.

13. Section *Ephebus.*—This huge section, so largely developed in the south-west, has actually more species in the Drakensbergen than in any of the other areas from George to the Zuurberg. The only species extending over the south-west and found in all the areas under consideration, *E. caffra*, is fairly constant. Only one variety has been described in the 'Flora Capensis'. None of the three purely eastern species are closely allied to it. They are placed without comment in the 'Flora Capensis' between south-western species. *N. Varderi*, L., Bolus, which is quite isolated amongst eastern species, is only known from one valley near Grahamstown.

14. Section *Ceramia.*—This section is absent between Port Elizabeth and the Drakensbergen. *E. trichoclada* occurs in Natal at an altitude of 4,000 ft. Bolus and Guthrie make the following remarks on this species ('Flora Cap.', iv. 147): 'A species singularly interesting as being an outlier from the great central home of heaths and as closely resembling *E. leptoclada*, from the station of which it is separated by 800 miles, while both appear to be rare.'

17. Section *Pyronium.—E. drakensbergensis* stands between *E. deliciosa* and *E. decipiens*; the former extends from Riversdale to Van Stadens, the latter from Knysna to the Zuurberg and Roschberg.

18. Section *Orophanes.*—The two species on the Drakensbergen are very isolated in their distribution; one, however, namely *E. sitiens*, occurs also in the Stellenbosch and Caledon divisions, and is unknown from the intervening distance of about 800 miles.

20. Section *Pachysa.*—Two species on the Drakensbergen, one of which extends westwards to the mountains near Queenstown, none on the Zuurberg or near Port Elizabeth. These two species are placed separately in the 'Flora Capensis' between south-western species.

22. Section *Chlorocodon.—E. Woodii*, Bol. (closely allied to *E. hispidula*, section *Arsace*), found on the Drakensbergen, the Houtbosch in the

Transvaal, and westwards to the mountains near Graaff Reinet, is otherwise only found on the Cedarberg Range, Clanwilliam. *E. maesta*, Bol., though absent from the Zuurberg, has a fairly continuous distribution in suitable localities from the Knysna to the Drakensbergen.

23. Section *Arsace* has four species on the Drakensbergen and one in Tembuland at 4,000 ft., namely *E. leuca* (var. γ). Other varieties are found westwards as far as the Caledon division. *E. hispidula*, L., is not found between Natal and the George division. *E. copiosa*, Wendl., widely spread in the south-west, does not occur east of Van Stadens until we come to Griqualand East. *E. ebracteata*, Bol., from the Mont-aux-Sources, has its nearest ally, *E. salax*, in the Stellenbosch division.

24. Section *Pseuderemia.—E. solandra*, Andr., was only known to Bolus and Guthrie from the George division and Van Reenen (Natal). A specimen from the Zuurberg, near Grahamstown, with undeveloped flowers, may belong to it.

30. Section *Geissostegia.*—This section is not recorded east of the Knysna division. An undescribed species is found on the Zuurberg, near Grahamstown.

31. Section *Elytrostegia.—E. glumaeflora* is one of the few species which has a distribution outside the south-west region at very low altitudes to the neighbourhood of East London. It rises to 3,000 ft. at Stutterheim, but already on the Zuurberg it ascends to 2,500 ft.

32. Section *Apoecus.*—Only two species are placed in this section by Guthrie and Bolus. One is confined to the Zuurberg, near Grahamstown, and the Amatolas (and adjoining mountains); the other, a very variable plant, continues this distribution north-eastwards to the Drakensbergen.

33. Section *Lamprotis.—E. Westii*, L., Bol., and *E. Simonsii*, L., Bol., are found at high altitudes on the Drakensbergen; all other species of the section are south-western, none east of Van Stadens.

36. Section *Trigemma.*—Again there are two species in this section known from the Drakensbergen (*E. Thodei*, G. and B., and *E. lasiocarpa*). No other species of this section is found east of the Knysna division.

38. Section *Eurystoma.—E. Wylei*, Bol., from the Giants' Castle, Drakensbergen (7,500–9,000 ft.), is allied to *E. floccifera*, Zahlbr. (= *E. floccosa*, Bartl. non Salisb.), which is only known from the Caledon and Robertson divisions.

39. Section *Melastemon.—E. cubica*, L., is not found between Natal and Van Stadens. The Natal specimens are placed under a distinct variety of this variable species.

40. Section *Gamocalamys.*—There is no species between Grahamstown and Natal. *E. natalitia*, Bol., connects this section with the section *Arsace.*'

Most of these remarks were excerpted by me some time ago, without any idea of proving or disproving a theory. They show, however, plainly in the first place that when we come to bed-rock of Willis's 'Age and Area theory' by comparing a number of closely allied species of the same ecological type, the present distribution of the South African species of *Erica* does not fit in with a mechanical rule such as Willis thinks he has discovered as governing the distribution of plants. The species which occur at widely separated stations or closely allied species in a similar position, both of which will be found in these brief remarks, are particularly interesting, as previously pointed out, because if they disappeared from either of their distant stations their whole position in a scheme of distribution would be completely altered, and this must frequently have happened in other species.

When we come to the tropical African species we again find little to help us in gauging age by area. Ten species have been described from tropical Africa. They are all found in the eastern mountains of the equatorial belt, with the exception of *E. arborea*, which is also found in Abyssinia, the Canary Islands, the Mediterranean region northward to Dalmatia and the Tyrol. None are identical with South African species. Engler says of *E. Princeana*, Engl., from northern Nyasaland, that it resembles superficially *E. Oatesii*, Rolfe (subgenus *Syringodea*, sect. *Dasyanthes*). All others appear to belong to the subgenus *Eu-Erica*, three from Nyasaland belong to section *Ceramia*, two belong to section *Hermes* (Nyasaland and eastern Rhodesia), three (incl. *E. arborea*, L.) to section *Arsace*, and one from Nyasaland to section *Pseuderemia*. The remaining species of *Erica* are mostly found in South, Central, and West Europe (as far as Norway). All seem to belong to the subgenus *Eu-Erica* (as defined by Bolus and Guthrie in the 'Flora Capensis', vol. iv). Some belong to sections which are not found in Africa, others belong to the following sections: *Pyronium* (absent from tropical Africa; includes a South European species, *E. umbellata*, L., closely allied to the South African species, *E. deliciosa*, Wendl.), *Gypsocallis* (absent from tropical Africa), and *Arsace*.

We may be justified in concluding from these facts of distribution that *Pyronium*, *Gypsocallis*, and *Arsace* represent very ancient groups of *Erica*, but we dare not go farther and conclude that they are the most ancient ones. Since *Pyronium* and *Gypsocallis* are absent from tropical Africa we have here, on a bigger scale than before in the genus *Erica*, a hint that isolated distribution should make us very cautious in drawing conclusions as to age from present-day distribution. If either of these sections were to disappear from remote stations, as they must have disappeared from intermediate stations (unless they have been evolved by convergence), they would now occupy a comparatively small territory and would appear to be comparatively new groups.

Before further commenting on these facts of distribution we must glance at the distribution of African genera allied to *Erica*, of which no less than eighteen are distinguished by N. E. Brown in the fourth volume of the 'Flora Capensis'. I think there will be a general consensus of opinion that these are descended from *Erica* (like the genus *Bruckenthalia*, Reichb., which occurs in the Balkans. The South African genus *MacNabia*, Benth., has been rightly included by Bolus and Guthrie in *Erica*). Only three of these: *Phillippia*, Kl., *Ericinella*, Kl., and *Blaeria*, L., extend beyond South Africa. *Phillippia* occurs in South-west Cape Colony (west of George), near Graaff-Reinet, in Angola, Nyasaland, and generally east tropical Africa. Engler has given a survey of thirteen species in 'Bot. Jahrb.', xliii. 466, and Spencer Moore has described two in 'Journal Linn. Soc. (Bot.)', xl, 1911. One species occurs near sea-level on the islands of Mafia and Pembu, a most unusual adaptation to real tropical conditions of African Ericaceae. There are also a number of species on the Mascarene Islands.

Ericinella is only a small genus. It does not reach South-west Cape Colony. In South Africa it is only found here and there on the range of mountains stretching from the Amatolas to the neighbourhood of Graaff-Reinet. It is represented on the mountains of east and west tropical Africa and in Madagascar.

Blaeria is in South Africa confined to the south-west coast region. Two species are known from Angola (one of these also occurs in Nyasaland); one has a wide distribution, being found on the Cameroons Mountain, summit of Clarence Peak, in Abyssinia, and on Kilimanjaro. In addition, Engler has described (in 'Bot. Jahrb.', xliii) five species from the mountains of equatorial Africa.

The huge gaps in the distribution of these derived genera are particularly noteworthy. If we knew the genus *Erica* and its allies only, we might come to the conclusion that it has dwelt longest in South Africa, and that all species outside South Africa are outliers. Thus we would assume that the development has taken place in a northward direction. This assumption probably would to a certain extent be correct, but on considering the other *Ericaceae* we see that the original current of distribution has been in just the opposite direction. However, no 'Age and Area theory' can disentangle these two opposite currents except in the vaguest sort of way. Willis ('Ann. of Bot.', xxxv. 497), 'with the aid of a prediction of the bearings of Age and Area upon the subject', came to the conclusion, as regards the New Zealand flora, that the number of endemic species in any genus would rise 'gradually to a maximum at or near the point where the genus entered New Zealand in the first place'. Applying this prediction to the genus *Erica* in South Africa, this point would be a part of South-west Cape Colony west of George, where not only a large number of endemics are massed, but where moreover the greatest diversity owing to formation of subgenera and derived genera is to be found; but I fear no contradiction when I assert that it is certainly not the place where the genus *Erica* entered South Africa, or where it originated, and thus again Willis's theory breaks down. But before accepting this as final we will take a glance at the distribution of *Ericaceae* generally and of allied orders.

Clethraceae, with only one genus, *Clethra*, L., found in tropical and subtropical parts of both hemispheres (known also from the Tertiary in amber), but mainly in the Northern Hemisphere, in Africa at Madeira. It has a choripetalous corolla (which is also found amongst *Ericaceae-Rhododendroideae*) and possesses various other characters which may be looked upon as primitive.

Pirolaceae are north temperate, extending to the arctics. Allied to *Ericaceae-Rhododendroideae-Ledeae.*

Lennoaceae, found only from S. California to central Mexico. They are all root parasites.

Epacridaceae, found in Australia, Tasmania, New Zealand, New Caledonia, Hawaiian Islands, southernmost part of South America, Malay Archipelago to India. They are most nearly allied to the *Ericaceae-Ericoideae,* and may be derived from them. They are haplostemonous (or even with further reduction in the androecium); they have free sepals; filaments are usually not free, but to a certain extent united with the petals. The family shows other peculiarities which preclude the derivation of the *Er.-Ericoideae* from them, and thus it throws no light on the origin of this sub-order and of *Ericaceae* generally.

Diapensiaceae are a small northern family, circumpolar, chiefly arctic alpine, extending southwards to Scandinavia, the Himalayas, Tibet, and Japan. A most important family in so far as it shows relations to *Clethraceae*, *Pirolaceae*, *Ericaceae*, *Epacridaceae*, and even *Primulaceae* (according to some botanists also to other gamopetalous families).

Ericaceae.

1. Section *Rhododendroideae.*—Northern, circumpolar to subtropical parts. In Africa only on the Azores.

2. Section *Arbutoideae.*—Northern, circumpolar, but extending southwards to the tropics. As far as Africa is concerned there is the genus *Agauria* (which also occurs in Madagascar and Mauritius). It is a small genus found on the mountains of Angola (where also the allied endemic monotypic genus *Ficalhoa*, Hiern, occurs), the Cameroons, and Fernando Po.

3. Section *Vaccinioideae.*—Northern, circumpolar, extending southwards in both hemispheres to the tropics. Only one genus poorly represented in Africa, namely, by a species of *Vaccinium* in Nyasaland and by another on the Drakensbergen.

4. Section *Ericoideae.*—These have all been referred to in connexion with our discussion of the distribution of the genus *Erica,* except the monotypic genus *Calluna,* which is widely spread in Europe, especially on the

mountainous parts of western Europe, but also occurs (though rarely) on the Atlantic coast of North America and Newfoundland.

It will be seen that the *Vaccinioideae* and *Arbutoideae* actually cover a wider area than the *Ericoideae*, but nobody will, I think, conclude from this fact that they are older than the *Ericoideae*. Contrary to Willis's rule, we may be justified in drawing the conclusion from a consideration of these areas that the *Ericoideae* and even the genus *Erica* have had their origin in the Northern Hemisphere, but he would be a bold man who would go farther, and from the facts of distribution alone deduce the relative ages of genera, subgenera, and species. In the absence of geological evidence we are largely thrown back on such evidence as comparative morphology affords, and distributional data can only be used cautiously as a subsidiary help, but there is not, and can never be, a definite rule to connect 'Age and Area'.

From the first, when Willis's 'Age and Area theory' was promulgated, I have looked upon it with suspicion. I have tested it in numerous instances (with South African and other plants) not mentioned in this paper, which I did not wish to extend unduly. There was also the same result, namely, that it is not a guide to our understanding present and past distribution of plants. Where it appears to be applicable it resolves itself into the platitude that of two species equally endowed in the struggle for existence, the older will have a better chance under equal conditions to occupy a larger area; but since two species can hardly ever be so equally balanced, and since really equal conditions can never be met by them in their migrations, the result in the most favourable cases can only be approximately fulfilling the prediction of the theory. Moreover, we can never be sure *when* it may be applicable. All the valuable lessons which we have learned during recent years from the study of plant-successions are the best vindication of the soundness of Darwin's Theory of Natural Selection in the struggle for existence, and we are on safe ground in adhering to it, provided we do not lose sight of the fact that Natural Selection is not a creative force. It is 'the rudder by which the ship is steered'. It determines the course which plants take in their migrations; frequently it leads them on to shoals where they stick, frequently it leads them to destruction.

A Remarkable Development of the Sporophyte in Anthoceros fusiformis, Aust.

BY

D. H. CAMPBELL.

With eight Figures in the Text.

THERE has been a marked tendency of late, especially among British botanists, to question the validity of the antithetic theory of alternation of generations in the Pteridophytes, and considerable ingenuity has been shown in the efforts to prove essential homologies existing between gametophyte and sporophyte.

None of the theories proposed has seemed to the writer to be based upon positive evidence at all commensurate with that brought forward in favour of the antithetic theory. Especially striking are the numerous resemblances between the sporophyte of the Anthocerotaceae and that of the lower Pteridophytes.

The Anthocerotaceae constitute one of the most clearly defined families of plants known to the botanist; but their relationships with other forms are by no means so evident. The highly developed sporophyte, especially in the genus *Anthoceros*, has naturally invited comparison with that of the simpler Pteridophytes, and the writer has long felt that the marked similarities of structure are something more than mere analogies.

The gametophyte of the Anthocerotaceae is very simple in structure, and fairly comparable with that of the Marattiaceae or *Equisetum*. The single chromatophore found in most of the Anthocerotaceae is very much like that of many green algae and is unknown elsewhere among the Bryophytes. Together with the very simple structure, this indicates that the gametophyte of the Anthocerotaceae is perhaps the most primitive of all the Bryophytes.

Of the American species of *Anthoceros*, the common Pacific Coast *A. fusiformis*, Aust., has the longest sporophyte. This often reaches a length of two or three inches, the maximum recorded by Howe[1] being

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[1] Howe, M. A.: The Anthocerotaceae of North America. Bull. Torrey Bot. Club, xxiv, p. 17 (1898).

[Annals of Botany, Vol. XXXVIII. No. CLI. July, 1924.]

9 cm. The sporophytes are produced in large numbers, so that a mass of fruiting plants looks like a tuft of fine grass.

The writer, in August, 1923, received from Carmel, near Monterey in California, some specimens of this species in which the sporophytes greatly exceeded in size the maximum figure given by Howe, and were the largest that the writer has seen in many years' collecting of Anthocerotaceae in various regions. The largest specimens were 16 cm. (quite six inches) in length. The specimens were collected by Dr. G. J. Peirce, and were evidently survivors from the previous growing season. Ordinarily the plants dry up completely with the cessation of the rains in April or May, and, while the gametophyte revives with the autumn rains, the sporophyte perishes.

There were abundant rains in October, 1922, and it is probable that the sporophytes in question began to develop soon after, so that they may have been growing for nine months or more.

The specimens were collected in San Jose canyon, near Carmel. This canyon has a permanent stream, and is open to the sea fog which prevails along the central Californian coast during the summer. The plants were growing on low banks near the stream, where there also was a luxuriant growth of two familiar Liverworts, *Marchantia polymorpha* and *Fegatella conica.* It was clear that there was a sufficient supply of moisture to enable the Anthoceros to continue growth during the summer, instead of drying up as it usually does, and the sporophytes, which undoubtedly had begun their development some time during the previous autumn, were still actively growing in September when the writer visited the locality.

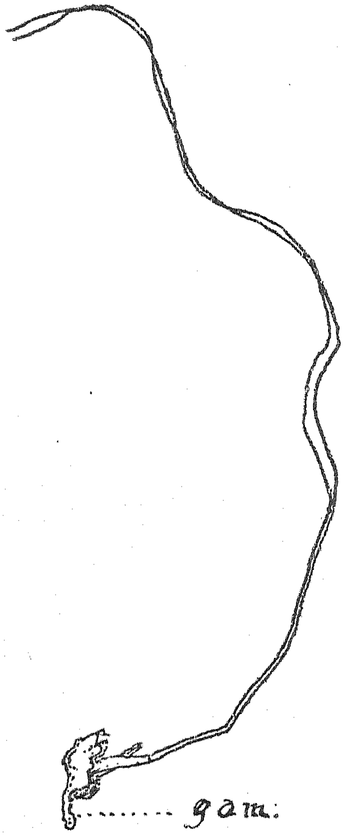

FIG. 1. Gametophyte (*gam.*) of *Anthoceros fusiformis*, Aust., bearing a very large sporophyte. Natural size.

A preliminary note, describing these remarkable specimens, has been published,[1] but the details of the structure, which show some most interesting and significant deviations from the normal sporophytes, seem worthy of more extended study.

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[1] Science, vol. lviii, pp. 307–8, Oct. 1923.

Since publishing the note the writer has learned that Professor C. J. Chamberlain had collected in Mexico an *Anthoceros* with remarkably long sporophytes. Dr. Chamberlain writes that the plants were collected in 1904, near Jalapa, growing on a railway embankment. He estimated their length as 'six or seven inches', but made no exact measurements, nor, apparently, was the species determined.

The material was given to Dr. W. J. S. Land, who later himself collected specimens in Mexico, and made a study of them, but has not published his results.

Whether this Mexican *Anthoceros* is the Pacific Coast *A. fusiformis* remains to be seen, but it is by no means unlikely.

When the Carmel specimens were compared with normal sporophytes of the same species collected at Stanford University, not only was there found a surprising increase in the length of the sporophytes, but they were noticeably more bulky, and an examination of the tissues showed some remarkable and interesting developments. The older portions of the capsule had developed spores of the usual form, and there was nothing to indicate that the early history of the sporophyte had not been entirely normal.

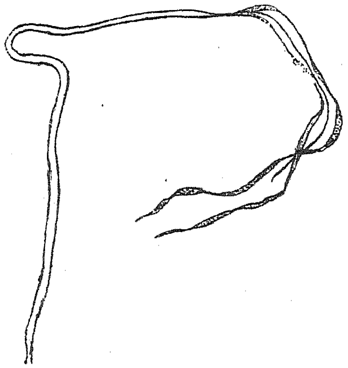

FIG. 2. Sporophyte, showing unusual development of the basal region and relatively short sporogenous portion. × 2.

The most significant thing about the enlarged sporophytes was the strong tendency towards the development of vegetative tissue. In some of these the basal region, for a length of 4½ cm., was still green, and in some few (see Fig. 2) there was a slight hypertrophy of the basal region, causing a marked distortion, and associated with a relatively short spore-bearing region.

A comparison of sections of the sporophyte where it had attained its full diameter shows that the enlarged sporophytes were from 0·6 mm. to 1·0 mm. in thickness, while the normal sporophyte had a diameter of only 0·5 mm. or less.

A cross-section near the base of the normal sporophyte shows the columella to consist of about sixteen cells arranged in an almost perfect square (Fig. 3, A). Surrounding the columella is a single layer of sporogenous tissue, the archesporium, outside of which are about three layers of cells, the outer of which later forms a very distinct epidermis.

Sections made higher up (Fig. 3, A) show a large lacuna surrounding the columella, which has become entirely detached from the other tissues, and has begun to dry up, the cells being somewhat shrunken and apparently quite dead (Fig. 3, C).

The sporogenous layer is differentiated into the spore mother-cells and sterile cells which lie free in the lacuna. The former undergo the usual tetrad division. Between the lacuna and the epidermis is the green tissue, consisting of about five or six layers of cells.

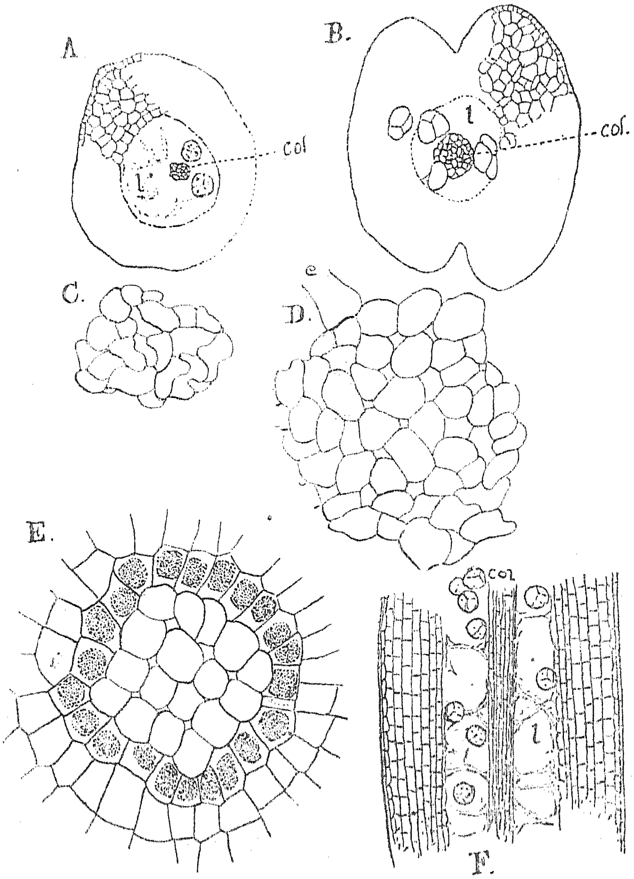

Fig. 3. A. Cross-section of a normal sporophyte of *A. fusiformis*, showing the large lacuna, *l*, and the isolated columella, *col*. × about 60. B. Cross-section of one of the larger sporophytes; the columella is notably enlarged, and connected with the cortex by sterile archesporial cells. × 60. C. Columella of the normal sporophyte. × 380. D. Columella of B. × 380. *e*, sterile archesporial cell. E. Cross-section of the base of a normal sporophyte, showing the single layer of archesporial cells surrounding the columella. × 380. F. Longitudinal section of a sporophyte, much like that shown in B, the columella joined to the cortex by the sterile archesporial cells. × about 60.

When sections of the enlarged sporophytes are compared with those from the normal ones, very marked differences are evident.

In some of the less developed ones there was still present a conspicuous lacuna surrounding the columella; but in no cases seen was this so large as in the normal sporophyte, and in the larger sporophytes it had nearly or quite disappeared.

Even where the lacuna is developed and normal spores formed (Fig. 3, B, F), the columella, which is much larger than normal, remains connected with the green tissue by means of the sterile cells of the archesporium, and the cells of the columella remain alive and functional. The columella is nearly twice the diameter of that of the normal sporophyte, and in longitudinal section it appears as a stout strand of elongated cells, highly suggestive of a simple vascular bundle.

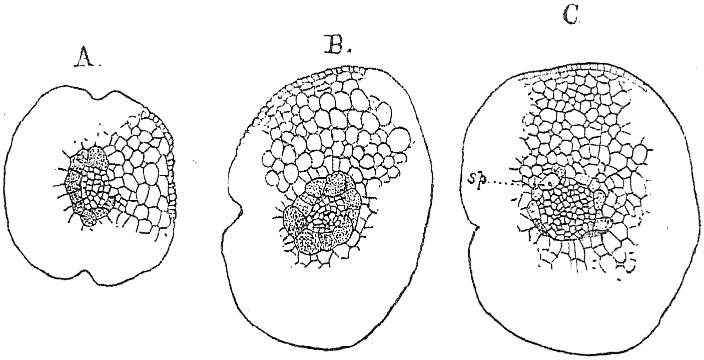

FIG. 4. A, B. Two sections from the base of the sporophyte shown in Fig. 2. A is nearer the base. The archesporial tissue is shaded. C. Cross-section of a large sporophyte in which the sporogenous tissue (*sp.*) was almost completely abortive. All figures × about 60.

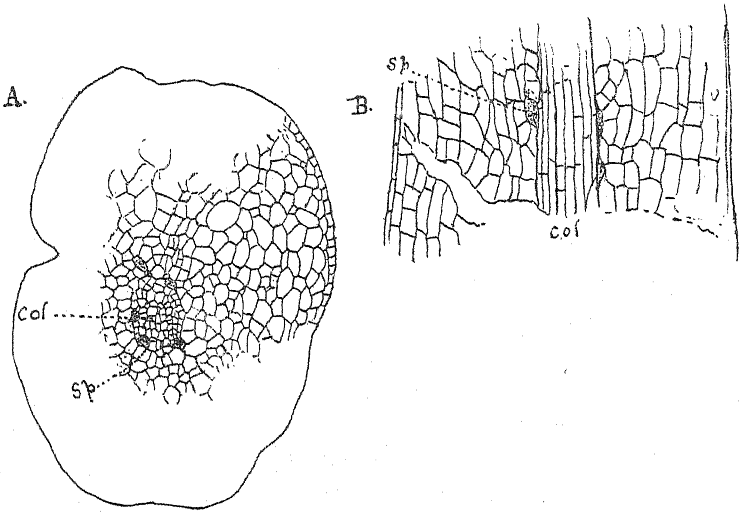

FIG. 5. A. Cross-section, B. Longitudinal section, of the thickest sporophyte that was found. *col.*, columella; *sp.*, sporogenous tissue. × about 60.

With the reduction in the size of the lacuna there is a corresponding increase in the amount of green tissue, and this is still more evident in some of the larger sporophytes, in which the lacuna quite disappears and the greatly enlarged columella is separated from the cortical green tissue, which is more than twice as thick as that of the normal form, only by an imperfect layer of quite abortive sporogenous cells.

Fig. 4, C, and Fig. 5 show sections of two of the large sporophytes, in which the very conspicuous central strand is to a great extent in direct contact with the innermost layer of the cortex, the sporogenous tissue being reduced to a few scattered, disorganized cells, these large sporophytes apparently having quite lost the power of spore-production in their younger portions. The basal part of these sporophytes is practically composed of a central stele, enclosed by a massive layer of green tissue, which may perhaps be called a cortex. While the axial stele shows no differentiation comparable to that in the true vascular plants, still there is little question that it is a true conducting tissue, and both morphologically and physiologically comparable to the simple axial stele of the Psilotales, or that of the earliest stages of such a fern as *Ophioglossum*.

Fig. 2 shows an unusually interesting case. The stoutish, somewhat distorted green basal region, some 4 cm. in length, terminates abruptly in the apparently normal open capsule, with its two valves and slender columella.

Fig. 4, A, B, shows two sections of this specimen made near the base. No lacuna is present, and although there is a considerable development of archesporial tissue it is abnormal in appearance, and it is very unlikely that any spores would be formed. The condition in this plant, as regards the sporogenous tissue, is transitional between the normal spore formation, shown in Fig. 3, B, and the complete abortion of the sporogenous tissue, shown in Fig. 4, C, and Fig. 5.

Fig. 5 shows cross and longitudinal sections of the thickest sporophyte that was examined. This was about one millimetre in diameter, and, except for a few scattered and quite disorganized sporogenous cells in contact with the very conspicuous central columella, was composed entirely of vegetative tissue. The columella is quite twice the diameter of those specimens in which a lacuna and normal spores were formed in the basal region (see Fig. 3, B).

THE FOOT.

The foot of the enlarged sporophytes shows an increase in size compared with that of the normal sporophyte. In the latter, the bulbous foot (Fig. 6, A) has a superficial layer of short, rhizoid-like absorbing cells (Fig. 6, D), and there is no essential structural difference to be noted in the foot of the enlarged sporophytes. The size of the foot is correlated with the amount of vegetative tissue in the rest of the sporophyte. Where this is

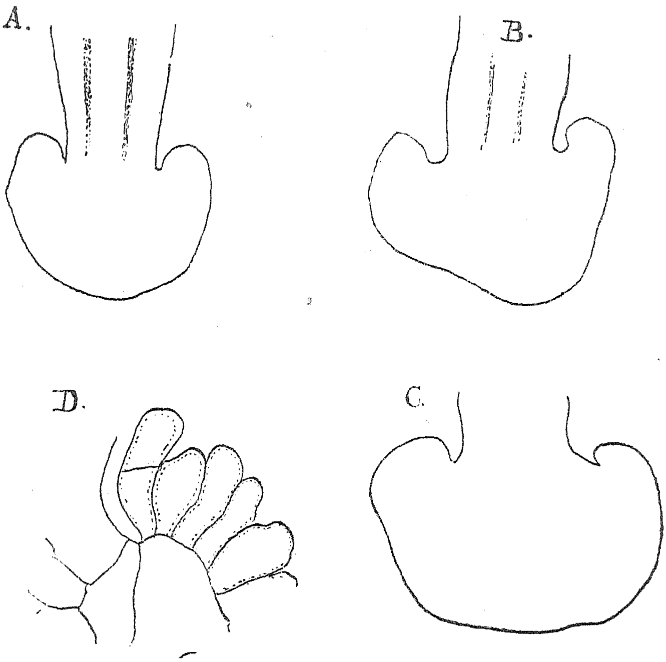

FIG. 6. A. Outline of the foot of a normal sporophyte. B. Foot of one of the less developed of the larger sporophytes, having lacuna and normal spores. C. Foot from a larger specimen. D. Superficial rhizoid-like cells of A. × 380. A–C × about 60.

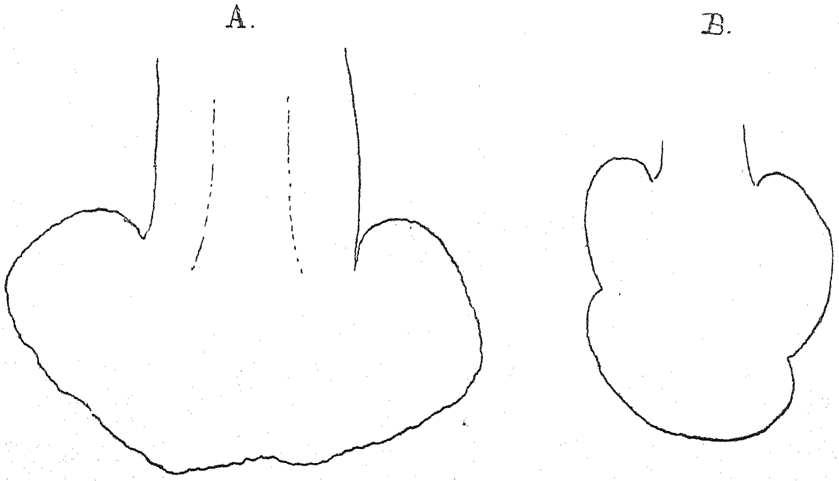

FIG. 7. A. The largest foot that was found; the lower surface was exposed, and probably absorbed water directly. × about 60. B. Foot of a large sporophyte, showing a conspicuous basal protuberance. × about 60.

relatively small, owing to the presence of a lacuna and normal spore-production, the foot is only slightly larger than in the normal sporophyte (Fig. 6, B); but in the larger sporophytes the increase in size is very marked (Fig. 6, D; Fig. 7). In the largest specimens that were examined (Fig. 7, A), not only was the foot very much enlarged, but the lower surface was almost completely exposed, due apparently to the disorganization of the adjacent gametophytic tissue. It is very probable that the foot in this case could absorb water from below without the intervention of the gametophytic tissues, thus rendering the sporophyte nearly or quite independent.

Fig. 7, B, shows an interesting case where there was a conspicuous protuberance growing from the lower surface of the foot, suggesting a root-like organ.

In most of the largest specimens examined, the gametophytic tissues in contact with the base of the sporophyte were discoloured and more or less collapsed, and it seems very likely that these large sporophytes had become practically independent of the gametophyte, and reached a condition comparable to that of the young Pteridophyte after it has established its first root. As will be seen from Fig. 1, the gametophyte is very small compared to the greatly developed sporophyte.

EVOLUTION OF THE SPOROPHYTE OF THE ANTHOCEROTACEAE.

The Anthocerotaceae offer a most interesting case of the progressive specialization of the sporophyte within a single order. *Notothylas* (Fig. 8, C), whose sporophyte is usually only 1–2 mm. in length, shows a comparatively large amount of sporogenous tissue, with a small columella; in *Dendroceros*, in which the sporophyte may reach a length of about a centimetre, the sporogenous layer, except at the apex, is but a single layer of cells. The sporogenous tissue is least developed relatively in the typical species of *Anthoceros*, where the sporophyte, as we have seen, reaches its maximum development. Rivalling *Anthoceros* in the size of the sporophyte, but with a much greater development of the sporogenous tissue and an absence of stomata, is the genus *Megaceros* (Fig. 8, B).

In *Anthoceros*, as a rule, the archesporium throughout is single layered; but in a common California species, *A. Pearsoni*, it is two-layered,[1] in which respect, as well as in a not infrequent doubling of the chromatophores in the gametophyte, there is a suggestion of *Megaceros*.

In both *Notothylas* and *Megaceros* the increased amount of sporogenous tissue in the apical region is worthy of note.

That the normal sporophyte of *Anthoceros* more nearly approaches a condition of independence than is known elsewhere among the Bryophytes has long been recognized; and the writer has from time to time made experiments to demonstrate how far they could be kept alive when severed as completely as possible from the gametophyte. While it cannot be said that any of these experiments were conclusive, it was nevertheless possible, by carefully cutting out the young sporophytes from the gametophyte and planting them in sterilized earth, to keep them alive for a considerable period—in several instances for three months. They made very little growth, but ripened perfectly normal spores in a number of cases.

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[1] Campbell, D. H.: Mosses and Ferns, 2nd edition, Fig. 72.

The specimens from Carmel, however, show that under certain conditions the sporophyte of *Anthoceros fusiformis* may become nearly or quite independent of the gametophyte, and thus approximate very closely to the hypothetical ancestors of the Pteridophytes.

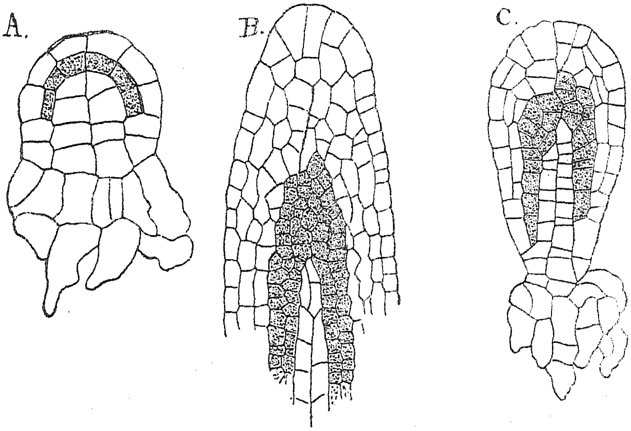

FIG. 8. A. Section of very young sporophyte of *Anthoceros Pearsoni*, Howe. B. Apex of a somewhat older sporophyte of *Megaceros Tjibodensis*, Campbell. C. Young sporophyte of *Notothylas orbicularis*. The sporogenous tissue is shaded.

COMPARISON WITH THE RHYNIACEAE.

Perhaps the most interesting fact revealed by a study of these peculiar sporophytes of *Anthoceros* is the extraordinary resemblance they bear to what are supposed to be the oldest known vascular plants.

The discovery of Kidston and Lang,[1] in the Lower Devonian of Scotland, of most perfectly petrified remains of vascular plants of extreme simplicity, *Rhynia* and *Hornea*, must rank as one of the most important in the history of palaeobotany.

The structure of these plants was preserved in a most perfect condition, as is sufficiently evident from the beautiful micro-photographs illustrating the memoirs.

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[1] Transactions of the Royal Society, Edinburgh, vol. li, pt. iii, 761–83 (1917); ibid., vol. lii, pt. iii, pp. 603–27 (1920).

They were slender leafless plants of small size, some specimens of *Rhynia Gwynne-Vaughni* hardly exceeding in size the largest specimens of *Anthoceros*. They grew close together in a manner suggesting the crowded sporophytes of *Anthoceros fusiformis*, the upright shoots arising from slender branching rhizomes. Some of the shoots were only a millimetre in diameter. A larger species, *R. major*, had stems up to six millimetres in diameter.

The shoot may be sparingly branched dichotomously, and in *R. Gwynne-Vaughni* has small protuberances which may develop into adventitious branches; but in *R. major* and *Hornea* the surface of the shoot is quite smooth, as in *Anthoceros*.

The cylindrical stem had an axial stele composed of a core of tracheary tissue surrounded by undifferentiated phloem, the rest of the stem being composed of (probably chlorophyllous) parenchyma, the transverse section, except for the tracheides of the stele, being almost identical with that of the largest of the *Anthoceros* sporophytes. In the smallest specimens of *R. Gwynne-Vaughni* the central tracheides were only one or two, seen in cross-section.

The shoot in both *Rhynia* and *Hornea* terminated in a single sporangium, that of the former elongated and pointed, and very suggestive of *Anthoceros*. In *Hornea* it was shorter, and the sporogenous tissue formed a thick dome-shaped layer surrounding a short columella, as in *Sphagnum*, but it also suggests the apical region in *Notothylas* or *Megaceros*. (See Fig. 8.)

The rhizome in *Hornea* is a massive lobed body which has been compared to the 'protocorm' of some species of *Lycopodium*, but is very suggestive of such a lobed foot of *Anthoceros* as that shown in Fig. 7, B. Unlike the root-like rhizome of *Rhynia* the 'protocorm' of *Hornea* is composed entirely of parenchyma, and is structurally identical with the foot of *Anthoceros*.

While Kidston and Lang recognize the obvious resemblances between *Anthoceros* and these ancient Pteridophytes, they hesitate to accept a real relationship between them.

However, in view of the extraordinarily close resemblances of the large sporophytes of *Anthoceros* discussed in the present paper, it seems to the writer that we are justified in assuming a derivation of the Rhyniaceae either directly from Anthocerotaceae or from forms closely related to them.

CONCLUSION.

That the sporophyte of *Anthoceros fusiformis* can under certain conditions become practically independent is amply shown by the behaviour of the plants under consideration; and the ability of these plants to develop photosynthetic and conducting tissues commensurate with their needs is extremely significant.

When, moreover, it is found that the earliest known Pteridophytes can be directly compared with existing Anthocerotaceae, we may fairly conclude that the behaviour of these remarkable plants of *A. fusiformis* is a very strong argument in favour of the theory that the sporophytes of the Pteridophytes are directly derived from those of *Anthoceros*-like ancestors.

STANFORD UNIVERSITY,
CALIFORNIA.

On a New Species of Tempskya from Montana: Tempskya Knowltoni, sp. nov.

BY

A. C. SEWARD, D.Sc., F.R.S.

With Plates XVI and XVII and three Figures in the Text.

IN September 1922 I received from my friend Dr. F. H. Knowlton, of the United States Geological Survey, an exceptionally well-preserved specimen of the problematical genus *Tempskya*, a Fern characteristic of certain Lower Cretaceous floras. He very generously invited me to undertake its examination and description: an invitation which I gratefully accepted. The fossil (Fig. 1, Pl. XVI) was found in 1908 by Mr. Albert C. Silberling in the Musselshell valley about 10 miles south-east of Harlowtown, Montana, and regarded by him as a caudal spine of *Stegosaurus*. A reference to the photograph, reproduced rather less than natural size in Fig. 1, enables one to sympathize with the collector's mistake. There is some uncertainty as to the exact geological horizon: it is either from the upper part of the Kootanie formation or from the lower part of the Colorado group. 'It is certainly', Dr. Knowlton tells me, 'one or the other, and is probably Kootanie.'

The Kootanie formation,[1] which was named by the late Dr. G. M. Dawson [2] in 1885 after a tribe of Indians, is represented over an area of about 3,000 square miles along the axis of the Rocky Mountains in Alberta and British Columbia; it occurs also in Montana and Wyoming. The strata are of freshwater origin and have yielded over eighty species of plants which do not include any Angiosperms.[3] The Kootanie flora agrees closely with the oldest Cretaceous floras of Europe, especially with those frequently spoken of as Wealden. Several Kootanie plants are represented by identical or closely allied species in the oldest Cretaceous flora of Greenland.

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[1] Pirsson and Schuchert (1920), p. 877; Berry (1911), p. 118, for lists of plants and references to literature.

[2] Dawson (1885), p. 2; see also Ward (1905), p. 277.

[3] Berry (1911), p. 118.

[Annals of Botany, Vol. XXXVIII. No. CLI. July, 1924.]

The genus *Tempskya* was founded by Corda[1] on material obtained from different sources, but none of it was collected *in situ*. The specimen from which the type-species, *Tempskya pulchra*, was described was discovered by F. Tempsky in a boulder in the valley of the Elbe, which, Corda stated, probably came from Neupaka in Bohemia. The author of the genus described four species, all of which may have come from Lower Cretaceous rocks in Bohemia. *Tempskya*, as described by Corda, stands for stem-like masses of petrified roots enclosing among them larger axes referred by him to petioles; it was identified as a herbaceous Fern. We are familiar with some of the anatomical characters of the stems and petioles, but so far it has not been possible to connect the petioles with any of the known species of Lower Cretaceous fronds preserved as impressions.

The most complete account of the anatomy and habit of this genus of Ferns is by Dr. Kidston and the late Prof. Gwynne-Vaughan,[2] who described a new species, *Tempskya rossica*, from a conglomerate of supposed Tertiary age in the basin of the Karaganda river, Russia. It is probable, one would suppose, that the *Tempskya* was derived from some older formation. In this important paper the authors made many additions to previous knowledge, and showed that in *Tempskya* we have a collection of slender solenostelic stems associated, without any regularity of disposition, with an enclosing plexus of roots. The larger axes, interpreted by Corda as petioles and by some authors as larger roots, are now known to be stems bearing crowded petioles represented by meristeles of a horseshoe form enclosed within the tissues of the parent stem. Only one free petiole was found in the Russian specimen. Kidston and Gwynne-Vaughan describe the aggregate of roots and stems as a false stem which was 'probably more or less conical and narrowing upwards. At the top of the false stem the individual axes it contained must have stood out separately, though the lower portion of each individual axis no doubt possessed its own coating of adventitious roots.' A restoration of *T. rossica* drawn by these authors was subsequently reproduced by Dr. Marie Stopes,[3] who also gave a *resumé* of the specific characters.

In view of the fact that the literature on *Tempskya* has been summarized by more than one author[4] references are given here only to a selected number of published papers. In 1824 a weathered piece of stem from the Wealden beds of Tilgate Forest in Sussex was figured in a paper drawn up by the Secretaries of the Geological Society of London, Messrs. Stokes and Webb,[5] apparently based on a contribution by Mr. Mantell. In the 'Explanation of Plates' the specimen is named *Endogenites erosa*, the generic name being selected because of the superficial resemblance of the matted roots to the scattered bundles of a Monocotyledonous stem. In 1836 Fitton[1] recorded the occurrence of numerous examples of *Endogenites erosa* in Wealden beds near Hastings, and spoke of one specimen as 9 ft. long, 12 in. broad in the middle, and 4 in. thick. In 1845 Unger[2] renamed the English fossils *Protopteris erosa*, but later he believed them to be identical with *Tempskya Schimperi*, Cord. This designation has generally been adopted for the various examples of *Tempskya* from England, North Germany, and Bohemia; but, as Dr. Stopes has reminded us, the English specimens, at least, should be designated *Tempskya erosa*. Schenk[3] figured an imperfectly petrified specimen from the Wealden of North Germany, and interpreted the larger groups of vascular tissue associated with the roots, which we now know to be solenosteles, as portions of the vascular system of a Marattiaceous stem. Velenovsky[4] discussed at length the nature of the genus and described specimens from the Perucer (Lower Cretaceous) beds of Bohemia. In 1911 Mr. Berry[5] spoke of *Tempskya* material as abundant but poorly preserved in the Potomac beds of Maryland: he briefly described some portions of a large stem nearly 40 cm. long as a new species, *Tempskya Whitei*, but from the illustrations and preliminary account it is not clear on what distinguishing characters the species is founded. It is by no means unlikely that Berry's species is identical with the Montana plant. Kidston and Gwynne-Vaughan, whose account of the genus is based on excellent material, do not commit themselves to a definite statement on the affinity of this Cretaceous Fern. In a section of a *Tempskya* stem, probably *T. erosa* (the preservation is very incomplete), from the Wealden beds of Hastings Dr. Boodle[6] found a cluster of 200-300 spores associated with the adventitious roots. The spores were compared with those of the recent Schizaeaceous Fern *Trochopteris elegans* (= *Aneimia elegans*) and with some other living species: Dr. Boodle wrote, 'Though this type of spore-thickening is uncommon, it occurs in plants that do not seem to be nearly related to one another; and therefore the resemblance to *Aneimia* in spore-structure is insufficient, without the evidence of sporangial character, for referring the fossil spores to the neighbourhood of Schizaeaceae'. The preservation of the spore clusters among the matted roots does not necessarily imply that they were borne on the fronds of a *Tempskya*. The root shown in Fig. 21, Pl. XVII, is an illustration of the preservation of a foreign body in the false stem of this genus. In one of the sections described by Dr. Boodle, for the loan of which I am indebted to Prof. Farmer, I noticed that one of the groups of spores presents the appearance of a collection of sporangia, the walls of which are represented by dark rods derived from cell-contents. It is, however, impossible to make out the nature of the sporangia. The Abbé

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[1] Corda (1845), p. 81.

[2] Kidston and Gwynne-Vaughan (1911).

[3] Stopes (1915), p. 15.

[4] Seward (1894), p. 148; Stopes, loc. cit.

[5] Stokes and Webb (1824).

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[1] Fitton (1836). [2] Unger (1845), p. 107. [3] Schenk (1871), Plates XLII, XLIII.
[4] Velenovsky (1888), p. 23. [5] Berry (1911), p. 298, Plates XXXVII, XXXVIII.
[6] Boodle (1895).

Carpentier[1] has recently recorded the occurrence of pieces of the false stem of a *Tempskya* from Glageon in the north of France: some of the specimens reach a length of nearly 20 cm. with a maximum diameter of 15·0 cm. The fossils, which were found in a bed of clay that is in part derived from Wealden strata, are said to be very similar to *T. Schimperi* and *T. Whitei*. A sketch accompanying the description shows two branching solenosteles and the usual mass of roots. The French material does not furnish any fresh evidence of affinity.

DESCRIPTION OF THE SPECIMEN.

Fig. 1, Pl. XVI, shows the fossil as it was discovered: it is 33·5 cm. long; in the broadest part the major diameter is 6·5 cm. and at the obliquely fractured lower end 1·5 cm. The elliptical form in section may be due to slight compression. The surface appears to be waterworn; innumerable sinuous roots are represented by grooves about 1 mm. broad (Figs. 1 and 3, Pl. XVI), and occasional broader grooves, *s.*, mark the position of stems. Scattered among the overwhelming roots are slender, dichotomously branched, solenostelic stems (Fig. 9, Pl. XVI), 3–4 mm. in diameter, in each of which are usually two meristeles at different stages of development passing through the cortex or circumscribed by the tissues of a half-detached leaf-base (Figs. 10, 12, Pl. XVI; Fig. 25, Pl. XVII). The term 'false stem', used by Kidston and Gwynne-Vaughan in their account of *Tempskya rossica*, may appropriately be adopted on the ground that the specimen consists not of a single axis with a central vascular cylinder, but of a number of apparently independent axes, some connected with one another as twin branches of a dichotomy, others pursuing their own course and subdividing as they grew.

The preservation is on the whole remarkably good and in places almost perfect. Many of the cells contain dark contents which may well have been formed from starch, and in others the lumen is occupied by a froth-like reticulum.

Text-fig. 1 shows the approximate position of the transverse and longitudinal sections which have been examined. The small piece (6·5 cm. long) retained by Dr. Knowlton is indicated by the broken lines: a transverse section from this region was unfortunately broken in the post. There is no doubt that the narrow end is the oldest portion; the stems embedded in the enveloping roots increase in number as one passes towards the broader end, and this is, in part at least, due to their repeated dichotomy, as a comparison of adjacent sections clearly demonstrates. There is a certain regularity in the orientation of the outgoing petioles in relation to the stems. In Text-fig. 2, which is a diagrammatic sketch of the section reproduced in Fig. 9, Pl. XVI, the direction of the straight lines drawn from several stems shows that the leaves are in the main on the upper side of the stems, or, at least, that they are not given off on all sides. As in the Russian species, *Tempskya rossica*, there is a definite dorsiventrality; roots emerge from the stems opposite the leaves (Text-fig. 2, R; Fig. 12, R, Pl. XVI), but in the American species the disposition of the dorsiventral stems is much more regular in relation to the position of the leaves and roots than in the Russian species.

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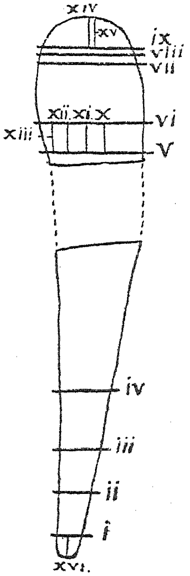
[1] Carpentier (1923).



TEXT-FIG. I. Diagram of the false stem of *Tempskya Knowltoni*, showing the approximate places where sections were cut.

The lowest section, i (1·3 × 1 cm.), includes only a single, very imperfectly preserved stem, and this was no doubt in a half-decayed state when petrifaction occurred. The rest of the section consists of a crowded mass of roots, most of which are in a more or less disorganized condition; in a few the xylem strand is well preserved, but in many the inner sclerenchymatous cortex encloses a central space from which the xylem has disappeared and has been replaced by a group of dark, oval bodies, possibly coprolites of some wood-boring organism (Fig. 24, Pl. XVII). The central region of some roots is occupied by a fine reticulum of dark lines on a light yellow ground simulating delicate parenchyma. Frequently, even in roots which have lost the xylem strand, the suberized endodermal membranes remain as a partially collapsed ring (Figs. 18, 19, *e*, Pl. XVII). The resistance to decay of the endodermis is interesting in relation to the recent work of Prof. Priestley[1] and the observations of Prof. Bower.[2] Photographs of roots showing the central region occupied by dark oval bodies and a delicate reticulum have already been published.[3]

In section ii (2·2 × 1·5 cm.) there are portions of two stems and, in addition, an imperfectly preserved and broken piece of dark sclerenchymatous tissue connected with two vascular ribs, *m.*, *m.*, which suggest the bifurcation of a leaf-axis (Fig. 6, Pl. XVI). It is noteworthy that in section i, and also in sections iii and iv, none of the stems are in a state of preservation which suggests the petrifaction of recently dead or of living organs.

In section iv there are four partially destroyed stems and a detached petiole. The larger section, v (5·5 × 3·8 cm.), includes about thirteen transversely cut stems. The majority of the stems show, in addition to remains of stelar tissue, one or two meristeles which, though not absolutely constant in their relation to the steles, exhibit a distinct tendency towards the upper surface of the mother-stem. In this section two of the stems have their tissues well preserved and give the impression of preservation before decay had begun: the others are usually broken and invaded by roots. The preservation of section vi is inferior to that of section v. In section vii there are over 20 stems, and, as in section v, many of them are forking. Section vii is reproduced in Fig. 9, Pl. XVI; it is 4·2 × 5·1 cm. in size. There are at least 24 stems or portions of stems scattered through the matrix of roots, and many of them are either forking or have recently branched. The diagrammatic sketch (Text-fig. 2) shows the position of the recognizable stems. Stem 14 is the only one which has its tissues intact and is free from invading roots: nearly all the others are more or less broken, their internal tissues having been disrupted by burrowing roots. Stem 14 must have been petrified very soon after the death of the plant, while practically all the others had undergone considerable decay. The orientation of the petioles, as shown by the curve of the meristeles in stems that are not too much disorganized to afford the necessary data, is indicated by the straight lines. Roots, R., are seen emerging from the outer cortex in several of the stems, and one root is seen at R in Fig. 12, Pl. XVI. Stems 1 to 4 are badly broken and very imperfectly preserved. The preservation of stem 5 is fairly good, but its tissues have been invaded by roots. Stem 6 is also much broken and includes several roots. Stem 7 is partially divided into two equal branches, and on the left-hand lower edge a root is being given off; the preservation of the tissues is very incomplete. A further stage in dichotomy is seen in stem 8: there are several invading roots within the outer cortex, and the steles are broken: a root is seen on the

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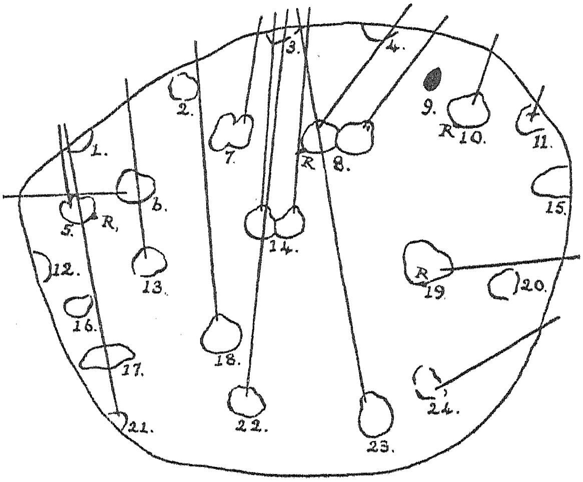
[1] Priestley and North (1822).

[2] Bower (1823), p. 186.

[3] Seward (1823), Plate XI, Figs. 1–3.



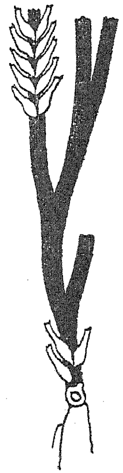
TEXT-FIG. 2. *Tempskya Knowltoni.* Diagram of the section reproduced in Fig. 9, Pl. XVI, showing the distribution of stems and the direction in which the leaves are being given off.

lower left-hand side. The dark patch of disorganized tissue, 9, may be the remains of a stem. Two roots are attached to the surface of stem 10, which is much broken. Portions of the xylem of stem 11 are preserved and roots are emerging from the middle of the lower surface. The preservation of stems 12 and 13 is very imperfect and the tissues have been largely replaced by roots. Stem 14 is shown on a larger scale in Fig. 10, Pl. XVI. Stems 15, 16, 17, are very incomplete. In stem 18 the stele is fairly well preserved, but invading roots are present. In stem 19 are also many roots. Stems 20, 22, 23, 24, are crushed and imperfectly preserved. A petiole only is seen in 21.

Though generally cut transversely the roots are occasionally seen in longitudinal section.

A comparison of the stems seen in section vii with those in section viii shows that dichotomy has been completed or advanced a stage farther. Stems 1 and 2 are both very imperfectly preserved, but they show signs of forking. Stem 7 is represented by two distinct branches, also stem 8. Stem 14 is divided into equal branches (Fig. 12, Pl. XVI). Stems 17 and 19 are dividing. In section ix there is only one stem among about thirty in which the preservation is at all complete.

Thus it would seem that in the elongated, obconical specimen there are enclosed several repeatedly forked stems, most of which were in a partially decayed state at the time of petrifaction, giving off two rows of leaves alternately right and left and, within certain limits, similarly orientated. The possibility of some of the dead stems having been displaced must be recognized, but the evidence is in favour of a certain regularity of disposition of the fronds which suggests an obliquely ascending and in part subterranean axis rather than a vertical column. In Text-fig. 3 an attempt is made to illustrate the external morphology of a stem: only a few leaf-bases are shown, and for the sake of clearness the roots are omitted, with the exception of two at the lower end. There is no indication of an absciss layer having been formed in the petioles.



TEXT-FIG. 3. Restoration of part of a stem of *Tempskya Knowltoni*, illustrating the dichotomous branching and the position of a few leaf-bases and roots.

Before discussing the affinity of *Tempskya* we must consider more fully the structure of the stems, leaf-bases, and roots. In its morphological features the Montana specimen agrees generally both with *Tempskya Whitei* and the European forms, but its superior preservation distinguishes it from all previously described representatives of the genus save *T. rossica*, a species from which it is distinguished by certain features which may or may not be worthy of specific rank. I venture to name the specimen after my friend Dr. F. H. Knowlton, whose researches have played a conspicuous part in the advancment of palaeobotanical science.

Stems.

The distribution of the stems and the orientation of the leaf-bases are shown in Text-fig. 2 (= section vii) and in Fig. 9, Pl. XVI. Figs. 10 and 12, Pl. XVI, show successive stages in the dichotomy of stem 14 of Text-fig. 2.

The xylem of the solenostele appears to consist entirely of scalariform tracheides without any associated parenchyma; there are no recognizable protoxylem strands (Fig. 20, Pl. XVII). Occasionally on the outer edge of the xylem ring there are smaller tracheides, like those described in *Tempskya rossica*, some of which are connected with the origin of roots. It is impossible to recognize the precise limits of the phloem, pericycle, and endodermis. The greater part of the outer ground-tissue of the stem consists of thick sclerous elements, and internal to this is a zone of thin-walled tissue characterized by the presence of black, spherical cell-contents. The centre of the stem is occupied by a mass of sclerous tissue with dark contents; the outer boundary of this central tissue is marked by a deposit of brown material which forms a conspicuous dark band in the sections (Fig. 10, *a*, Pl. XVI; Fig. 20, *a*, Pl. XVII).

The broad and flat-arched meristele is represented by a band of xylem much narrower than that of the solenostele; the ends are expanded, but most of the arch is very narrow and in places may be reduced to a single tracheide in breadth (Figs. 14, 26, Pl. XVII). The concavity of the petiolar strand is occupied by an extension of the central sclerenchyma of the main stele. The meristele is concentric: a group of protoxylem is seen at *px.*, Fig. 26, Pl. XVII, and there are indications of two or three similar groups on the inner edge of the arch. The petiole shown in Fig. 14, Pl. XVII (its longest diameter is 2·6 mm.), is still attached to a broken stem penetrated by roots. A band of dark tissue, which has not reached its full development, connects the two broad ends of the black, U-shaped outer cortex. Next the black outer cortex is a zone of thinner-walled tissue with black balls in the cells as in the corresponding tissue in the stem (Fig. 13, Pl. XVI; Fig. 26, *i.c.*, Pl. XVII), and on the inner boundary of this part of the cortex the cells have thicker walls and are partially obscured by irregular patches of a brown substance (the black band enclosing the vascular strand in Figs. 14 and 26, Pl. XVII). Next to the cells with brown patches is a layer of regular, flat cells, probably the endodermis, and between this layer and the xylem are some thin-walled elements representing phloem and pericycle. The central sclerenchyma shows beautifully preserved pit-canals. Fig. 6, Pl. XVI, shows two meristeles, *m.*, enclosed in a common, broken band of dark cortex: this may be a bifurcating rachis or possibly part of a stem with two leaf-traces, the stele and most of the ground-tissue having been replaced by invading roots.

Several longitudinal sections were cut between the transverse sections v and vi: the position of these and others is shown in Text-fig. 1. My thanks are due to Mr. Hemingway for the care and skill with which he made preparations on which this account is based.

The leaves are given off at frequent intervals, their bases being contiguous (Figs. 11, 13, Pl. XVI). In the section reproduced in Fig. 11 two leaf-bases on the right are cut at different levels; and it is noteworthy that at the base of the lower petiole, P′, where it joins the stem, the outer sclerenchyma appears to form a thick pad, S, a feature probably connected with mechanical requirements. At the apex of the longitudinal section reproduced in Fig. 13, Pl. XVI, the outer sclerenchyma forms a dome—no doubt because the section is not median in this region—and above this, not shown in the photograph, is a strip of parenchymatous tissue which may be part of a ramental scale; but its nature is doubtful. No ramenta have been recognized with certainty. Internal to the dark, outer cortex is a lighter band, *i.c.*, of thin-walled cells containing black, spherical balls, possibly formed from starch (cf. Figs. 20, 26, Pl. XVII).

Within the inner cortex, *i.c.*, is the darker stelar region enclosing a broken patch of central sclerenchyma. On the right-hand side of Fig. 13, Pl. XVI, are the contiguous bases of three leaves with some invading roots between them and the stele.

Fig. 2, Pl. XVI, shows part of a longitudinal section of a stem. The outermost tissue consists of elongated, sclerous cells with dark contents passing gradually into thinner-walled and shorter cells of the inner cortex. A space in the inner cortex is filled with dark brown, oval bodies uniform in size and too large to be merely released cell-contents. The darker patch in the middle of the upper edge of the photograph is due to the occurrence of cells with frothy contents surrounding the xylem of the stele, which is not included in the figure.

The stem near the centre of Fig. 9, Pl. XVI (Text-fig. 2, stem 14), is shown in Fig. 10, Pl. XVI; its longest diameter is 6 mm. The tracheides in the middle of the upper side of the right-hand stele are in oblique longitudinal section as if preparing to form a leaf-trace. Above is a leaf-base with a complete meristele. The stele on the left has one arm of a meristele still attached to it on the right-hand side of the foliar gap, and farther to the left the projecting point of the central ground-tissue indicates an early stage in the formation of another leaf-trace. Along the outer edge of the central sclerenchyma is a dark band, *a*, composed of cells with irregular patches of brown material (cf. Fig. 20, *a*, Pl. XVII); this is succeeded by a space containing remains of delicate tissue with dark cell-contents. The xylem, without any protoxylem groups, is about nine or ten elements broad: some of the tracheides have dark contents, but no definite proof of associated parenchyma has been discovered. Fig. 20 shows part of the xylem cylinder in another stem with some of the inner cortex, *i.c.*, on the outer face. Fig. 12, Pl. XVI, shows a further stage in the bifurcation of the stem seen in Fig. 10, Pl. XVI. The separate stems are 3 and 3·5 mm. in diameter. On the right-hand side, at R, a root is emerging from the outer cortex. An outgoing meristele is seen as a loop on the upper surface of the right-hand stele and a petiole-base on the left. The thin-walled inner cortex forms a dark band external to the lighter xylem and is separated by a space from the thick outer cortex. The other stem shows a loop-like meristele to the right and above it a leaf-base; to the left is a third meristele.

Roots.

By far the greater part of the specimen consists of a mass of branching and burrowing roots usually, though by no means invariably, following a vertical course; a few here and there are seen emerging from stems, but the majority occur as apparently independent organs invading in their course the tissues of stems and petioles. Fig. 25, Pl. XVII, shows a stem in which roots have replaced the tissues of the stele and form a ring between the central ground-tissue and the partially destroyed inner cortex.

The contrast between the large number of roots without any visible connexion with their mother-organs and the small number of which the origin is demonstrated is remarkable. Most of them must have come from stems or leaf-bases that are unrepresented in the specimen.

All the roots are diarch and about 1 mm. in diameter, or rather larger; the xylem strand, with two or three large tracheides in the centre, is usually surrounded by a space where the phloem and pericycle have been replaced by clear silica, and the tracheides are often encircled by a highly resistant brown ring of endodermal cell-walls (Figs. 17–19, *e.*, Pl. XVII). It is interesting, in relation to the discussion on the affinity of *Tempskya*, to note the occurrence of a very distinct suberized endodermis in the roots of *Aneimia adiantifolia*, very similar to that in the roots of the fossil. Some roots have lost the xylem, and the centre is occupied by a group of dark brown bodies that may be coprolites of a small insect or, in some cases, possibly escaped cell-contents. Entomologists whom I have consulted have not been able to identify the oval bodies with the activities of any known boring animal: no trace of any insect has been discovered. Attention has elsewhere[1] been called to the resemblance of these bodies to the supposed coprolites frequently found in tissues of Carboniferous plants. They are certainly not spores, but consist of finely comminuted plant-debris or dark masses of rounded cell-contents. Part of a branched root is seen in Fig. 24, Pl. XVII, cut through the sclerotic cortex, the cells of which show pit-canals very clearly. The central region of both the main root and the branch is occupied by similar dark bodies, but in the lateral root these are uniformly smaller. In some roots the centre is filled with a fine reticulum of delicate lines simulating parenchyma, though undoubtedly of mineral origin and the result of secondary crystalization.

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[1] Seward (1823).

A broad parenchymatous cortex of sclerotic cells is usually well preserved (Figs. 7, 17, Pl. XVI; Fig. 19, Pl. XVII), the thick walls penetrated by pit-canals. Less frequently an outer cortex of thin-walled, large cells is also preserved (Fig. 7, Pl. XVI; Fig. 19, Pl. XVII). The outer cortex is limited on the outside by two or three layers of rather smaller and stouter cells (Figs. 17, 19, *o.c.*, Pl. XVII), and it is these superficial cells which are often seen as a loose sheath round the more resistant inner cortex or as loops between adjacent roots, as in Fig. 6, Pl. XVI; Figs. 17, 25, Pl. XVII). No root-hairs have been detected, but in some sections of the English *Tempskya erosa* well-preserved hairs are a striking feature. The irregular contour of the outer cortex, seen in Fig. 7, Pl. XVI, is not an original feature; it is the result of unequal compression and distortion of the yielding tissue by the pressure of neighbouring roots. In Fig. 15, Pl. XVII, a lateral root is seen on its way through the sclerous cortex of the parent axis. Beyond the lower limit of the photograph the section cuts obliquely the xylem of the main root. The xylem of the lateral root is diarch and contains more tracheides than the roots shown in Figs. 17–19, Pl. XVII. In his description of the roots of *Aneimia phyllitidis*, Boodle[1] states that the base of the root-stele in the stem cortex is diarch, but the middle region of the xylem-plate is occupied by numerous irregularly placed tracheides, a description which is applicable to the lateral root seen in Fig. 15, Pl. XVII.

The immature root, a part of which is reproduced in Fig. 23, Pl. XVII, is a striking illustration of the excellent preservation of delicate tissue. Two protoxylem groups are clearly seen, and even at this early stage of development the position of the partially suberized endodermis, *e.*, is indicated by a series of dark, radially disposed cell-walls in sharp contrast to the more delicate and crushed membranes of other elements. The recognition of dark, presumably suberized, walls in the endodermal layer of this and other very young roots of *Tempskya* confirms Bower's statement that in roots the first indications of the characteristic features of the endodermis 'coincides very nearly with the appearance of the first tracheides'.[2] In the root shown in Fig. 23, Pl. XVII, the inner cortex is not completely thickened, and outside the region reproduced the broad outer cortex is clearly preserved. In older roots the endodermal walls are completely suberized and dark brown in colour; this is shown in the oblique section reproduced in Fig. 18, *e.*, Pl. XVII.

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[1] Boodle (1901), p. 385.

[2] Bower (1923), p. 187.

OTHER PLANT ORGANS AND TISSUES ASSOCIATED WITH THE STEMS AND ROOTS OF *TEMPSKYA*.

I. *Annuli of Fern Sporangia.*

Figs. 5 and 8, Pl. XVI. The groups of thick-walled cells shown in these figures occur among the roots of the false stem. In Fig. 8, Pl. XVI, between 20 and 30 cells are arranged as a circle enclosing a central area in which it is impossible to make out the structure with certainty, but there are indications of small cells; the cells of the ring have dark brown, thick walls rounded at the distal ends and lying in a slightly oblique position. The diameter of the ring is 0·34 mm. Fig. 5, Pl. XVI, shows similar cells resembling an annulus that has been partially unrolled: the length is 0·47 mm. It is probable that in these groups we have apical annuli of Schizaeaceous sporangia of the *Aneimia* type. Halle's statement,[1] that the annulus cells of *Ruffordia Goepperti* are the only portions of the sporangia preserved in some specimens described by him from Manchuria, lends support to the suggestion that Figs. 5 and 8, Pl. XVI, represent the annuli of sporangia borne on the *Tempskya* fronds. The annuli described by Halle contain about 25 cells and the diameter is from 0·25 to 0·30 mm.: the agreement with the American specimens is close. Similarly the annuli of the sporangia of *Aneimia adiantifolia* agree fairly closely with the cell-groups shown in Figs. 5 and 8, Pl. XVI, and it is noteworthy that the occurrence of several cells in the distal region enclosed by the thick-walled elements is a feature characteristic of *Aneimia*:[2] in *Lygodium* and *Schizaea* the apex of a sporangium is occupied by a single cell.[3]

II. *Araucarian Roots.*

Figs. 21, 22, Pl. XVII. Lying among the roots of *Tempskya Knowltoni* in some of the sections (sections vi–ix, xvi) is a larger root belonging to an entirely different plant which had bored its way into the false stem presumably below the surface of the ground. A slightly oblique transverse section of the invading root is shown in Fig. 21, Pl. XVII; the elliptical xylem-tissue in the centre (1·0 × 0·38 mm.) consists of regular series of secondary tracheides with two or three rows of alternate, contiguous pits on the radial walls (Fig. 22, Pl. XVII) and occasional, imperfectly preserved medullary rays. In the centre along the major axis the elements appear to be disposed as a bipolar band of primary xylem with narrower tracheides at the ends. Fig. 22, Pl. XVII, shows part of the secondary xylem of another specimen, identical in form and structure with that represented in

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[1] Halle (1921).

[2] Prantl (1881), Plate VII, Fig. 104.

[3] Bower (1823), p. 253.

Fig. 21, Pl. XVII, which was found at a much higher level in the *Tempskya*. The xylem is surrounded by a space, and beyond this is a loose, compressed cylinder composed mainly of serially arranged thin-walled cells which are undoubtedly of the nature of cork (Fig. 21, P, Pl. XVII). External to the cork there are traces of a fairly broad band of parenchymatous cortex. The root is most probably Araucarian; it agrees closely in the form of the diarch xylem and in other features with the roots of recent Araucarias.

ENGLISH SPECIMENS OF *TEMPSKYA*.

I am indebted to Dr. Kidston for the photograph reproduced in Fig. 16, Pl. XVII, of a compressed solenostele of *Tempskya erosa*. For the loan of this and other sections cut from a specimen from the Wealden beds of Brighstone in the Isle of Wight my thanks are due to Prof. Farmer, of the Imperial College. The length of the major diameter of the xylem ring is 5 mm., whereas the complete stem of the American specimen has a diameter of about 3·5 mm. The xylem is from 8 to 10 elements broad: no protoxylem groups nor xylem parenchyma have been recognized. Two meristeles are shown above the broader end of the solenostele and two other meristeles are in course of development. Apart from the xylem the preservation is very poor: the ground-tissue has been replaced by a dark reticulum which, especially in the centre, simulates parenchyma. The roots are diarch and appear to be identical in structure with those of the American Fern.

Fig. 4, Pl. XVI, shows part of a section, in the British Museum collection, labelled *Tempskya erosa*, but from an unknown locality: it was no doubt cut from an English Wealden specimen. The whole section, 3 × 2·2 cm., contains 13 badly preserved stems lying in a dense mass of diarch roots. One of the stems seen in Fig. 4 on the left is partially divided; in another stem at P a protuberance marks the position of a leaf-base. The tendency is for the leaves to be given off from the stems on the same side, though the orientation is not absolutely constant. The steles are approximately of the same size as those of the American species, but anatomical comparison is precluded by the very imperfect preservation of the English specimen.

HABIT OF *TEMPSKYA KNOWLTONI*.

In their general conclusions Kidston and Gwynne-Vaughan wrote as follows: 'The aggregate of axes with their root-packing must have stood upright. If they had grown horizontally the roots would not have run parallel with the stems, and again the leaf-bearing sides of all the stems would have pointed in the same direction, i. e. away from the soil. The whole aggregate of axes and roots undoubtedly reached a very considerable height and size. . . In all probability, then, the whole plant when full-grown had the appearance of a tall column consisting of a mass of stems and adventitious roots. This "false stem", as it may be called, was probably more or less conical and narrowing upwards'.[1] This view of *Tempskya* is expressed in the restoration to which reference has already been made. The authors add, 'The presence of a strictly dorsiventral symmetry in the stems becomes a matter of no little surprise. It is hardly possible to account for it otherwise than as a vestigial character on the assumption that the plant primitively grew with a creeping horizontal rhizome.'

An examination of the American specimen leads me to express an opinion different from that of Kidston and Gwynne-Vaughan. It has already been shown that the false stem (Fig. 1, Pl. XVI) was obconical, the broadest end being the youngest portion. We cannot tell to what extent erosion and weathering altered the original form, but the shape of *Tempskya Knowltoni* and, one may add, the shape of much larger specimens of the English species figured by Fitton and other authors does not favour the idea of a vertical column. The general, though not invariable, tendency of the roots to follow a vertical course does not, I venture to think, justify the assumption of an erect position. The mass of compact roots seen in the fossil was probably covered during life by a much looser felt of roots which grew into the soil, but unless the false stem were preserved *in situ* one could not expect to find any remains of the superficial absorbing organs. Moreover, hydrotropism as well as geotropism must be taken into account. In *Tempskya Knowltoni* the orientation of the leaves is much less irregular than in *T. rossica*. The occurrence of a well-preserved Gymnospermous root among the roots of the Fern is consistent with a subterranean position: there is no evidence that the *Tempskya* was dead at the time of its penetration by the foreign root.

The greater regularity in the position of the leaves on the stems of *T. Knowltoni* as compared with their irregular disposition in *T. rossica* may be largely a question of age. Several years ago a stem of *Dicksonia antarctica* was taken out of the ground for me at Kew, and my recollection is that the basal subterranean part of the plant was in an obliquely ascending position and obconical in form. It may be that *Tempskya* in its earlier stages of growth was at least in part below the surface of the soil and resembled *Dryopteris Filix mas* in the orientation of the fronds. The Montana specimen probably represents such a comparatively juvenile stage as I have described. At a later stage the false stem may have assumed a more or less vertical position, and when this happened the stems would no longer exhibit any regularity in the orientation of the fronds.

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[1] Kidston and Gwynne-Vaughan (1911), p. 14.

I believe that the available evidence supports the conclusion that the false stem of *Tempskya*, or at least that of *T. Knowltoni*, may best be compared with the obliquely ascending, and for the most part subterranean, stem of such a Fern as *Dryopteris Filix mas*. The orientation of the leaves (Text-fig. 2) suggests that only a portion of the *Tempskya* was uncovered. On mechanical grounds it is difficult to imagine *T. Knowltoni* growing erect.

A section of a stem of *Cyathea medullaris* figured by Bower [1] shows a bifurcate stem embedded in a mass of roots: it is quoted in illustration of a Tree Fern stem which depends for the support of its lower and thinner region upon adventitious roots. The stem of *Cyathea*, like that of *Tempskya*, increases in diameter from a narrow base upwards. There are, however, obvious differences between the two Ferns, and particularly the contrast between a stem with a single median vascular axis, occasionally bifurcating, and bearing radially disposed fronds and a false stem such as that of *Tempskya*.

Fitton stated that the large specimens of *T. erosa* were found in a horizontal position in the English Wealden strata, and that they were elongated, flattened bodies 'tapering at both ends'.[2] Mantell [3] quoted Brongniart as saying that *Tempskya* bears some resemblance to the base, or the short and almost subterranean stems of some recent species of Ferns that are not arborescent. It is also not easy to explain the transport of water by the roots to the living stems in the upper part of an upright columnar mass containing only a few decayed stems in its basal region. In the absence of a complete series of sections it is impossible to ascertain whether or not the much greater number of stems in the upper, broader portion is the result of repeated dichotomy of a single axis, but it is not improbable that the numerous stems in the upper part of the specimen were all derived from a single individual. If, on the other hand, the Fern were on the ground, or in part below the ground, the roots of any of the stems would be within easy reach of water.

THE SYSTEMATIC POSITION OF *TEMPSKYA*.

Referring to the genus *Tempskya* in the course of a general account of Wealden floras published nine years ago,[4] I wrote: 'While the balance of evidence is distinctly favourable to the inclusion of *Tempskya* in the Schizaeaceae, definite proof is lacking. We have no information as to the form of the leaves, and it remains to be discovered which, if any, of the numerous unattached Fern leaves from Wealden beds belong to the *Tempskya* stems.' Anatomically the species described in this paper does not add very much to the facts furnished by *Tempskya rossica.* Kidston and Gwynne-Vaughan stated that 'the affinities of the Tempskyas appear to lie with the Leptosporangiate Ferns, but beyond this their systematic position must at present be left uncertain. The anatomy, so far as it is at present known, does not furnish sufficient evidence to assign them to one order rather than to another. For instance, stems with a solenostelic vascular system are known to occur in the Schizaeaceae, Gleicheniaceae, the *Dipteris-Matonia* series, in *Loxsoma*, the Cyatheae, the Dennstaedtineae, and in certain series of the Polypodiaceae. In each case, except perhaps the Cyatheae, a horseshoe-shaped leaf-trace is associated with the solenostele. Referring to the absence of definite protoxylem groups in the stem xylem, the same fact is to be noted in certain species of all the groups mentioned except in *Dipteris*, *Matonia*, the Gleicheniaceae, and Cyatheae'.[1]

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[1] Bower (1823), p. 158.

[2] Fitton (1836), p. 137.

[3] Mantell (1833), p. 237.

[4] Seward (1914), p. 136.

We may now consider whether it is possible to produce any additional evidence in justification of my suggestion, in 1914, that *Tempskya* is probably related to the Schizaeaceae. With the exception of a few species (sub-genus *Aneimiorrhiza*) the stems of *Aneimia* are dialystelic, while those of other Schizaeaceous Ferns have either a simple protostele or a medullated protostele. The xylem of *Aneimia mexicana*,[2] one of the species with a creeping solenostelic rhizome bearing distichous leaves, is about five tracheides broad and has no definite protoxylem groups, nor is there any parenchyma interspersed with the scalariform elements. *Aneimia adiantifolia*, a Floridan species, is anatomically similar. The roots of the Schizaeaceae, like those of many other Ferns, are diarch, and in *Aneimia* the xylem-plate includes two large tracheides like those in the roots of *Tempskya*. *Aneimia* agrees closely with *Tempskya* also in the flat-arched meristele. The solenostele of *Gleichenia pectinata*[3] is distinguished by the presence of clearly defined protoxylem groups and by other features. The roots are usually tetrach. In the solenostelic New Zealand Fern *Loxsoma Cunninghamii*,[4] now assigned to a separate family, the Loxsomaceae, the xylem is three to four elements broad and, as in *Aneimia*, the smallest tracheides occur on the outer edge; but it differs from *Tempskya* in having a single row of leaves that are rather widely separated. Moreover, the occurrence of islets of parenchyma in the sclerotic cortex is a characteristic feature. The Fern *Lophosoria pruinata*,[5] which is nearly allied to the Cyatheae, has a solenostele in which there are no protoxylem strands; but the rhizome is often very bulky and the fully developed leaf-trace is unlike that of *Tempskya*. *Metaxya rostrata*,[6] a nearly related species, has a solenostele, but here also the meristele is not so simple as that of the fossil genus, and the stem of the recent Fern is stouter.

[1] Kidston and Gwynne-Vaughan (1911), p. 16.

[2] Boodle (1901).

[3] Boodle (1901^2).

[4] Gwynne-Vaughan (1901).

[5] Bower (1912).

[6] Bower (1913).

In his paper of 1903 Gwynne-Vaughan[1] gave a list of solenostelic Ferns known at that time. Some of them differ from *Tempskya* in the greater diameter of the rhizome, and in many of them xylem-parenchyma is a conspicuous constituent of the stele. Prof. M'Lean Thompson[2] mentions some solenostelic Ferns, but in none of them is the resemblance to *Tempskya* as close as is the solenostele of *Aneimia*.

On anatomical grounds it would seem reasonable to suggest that the solenostelic species of *Aneimia* show the greatest similarity to the Wealden genus.

If I am correct in identifying the groups of thick-walled cells shown in Figs. 5 and 8, Pl. XVI, as annuli of sporangia, their association with roots of *T. Knowltoni* lends considerable support to the view that the spores, compared by Boodle[3] with those of a Schizaeaceous Fern found in the false stem of *T. erosa*, were from sporangia borne on *Tempskya* fronds.

So far as I am aware, no recent species of Schizaeaceae is known in which the slender stems are embedded in a mass of roots, nor is there any living Fern with a false stem of the *Tempskya* type. The Javan Fern *Hemitelia crenulata*, described by Schoute[4] and compared by Kidston and Gwynne-Vaughan with *Tempskya*, offers certain interesting analogies, but, as the latter authors show, there are essential differences in construction.

The next point to discuss is the possibility of connecting the false stem of *Tempskya* with any of the Lower Cretaceous species of Fern fronds. There is no proof of any such connexion. Among widely distributed species founded on fronds from geological formations from which *Tempskya* has been recorded there are *Weichselia reticulata* (= *W. Mantelli*), *Cladophlebis Browniana*, *Onychiopsis psilotoides* and *O. elongata*, *Ruffordia Goepperti*, in addition to species of *Gleichenites*, *Hausmannia*, *Sphenopteris*, and *Taeniopteris*. The stem and petiole of *Weichselia*[5] differ widely from those of *Tempskya*; there is clearly no real affinity. The fronds known as *Cladophlebis Browniana* are practically always sterile,[6] but specimens were discovered in Peruvian Wealden beds[7] identical in habit with Northern Hemisphere forms bearing sporangia of the Schizaeaceous type, and were therefore transferred to the genus *Klukia*, which includes the more familiar *K. exilis* of Jurassic floras,[8] a species hardly distinguishable from *K. Browniana*. *Klukia* fronds differ in habit from those of any recent species of Schizaeaceae, but they bear sporangia identical in structure with those of existing members of the family. It is possible that *Klukia* fronds were borne on *Tempskya* stems, but the smaller rachis of *Ruffordia Goepperti* and the slender evidence of forking afforded by the imperfect specimen seen in Fig. 6 are points in favour of connecting the latter species with *Tempskya*. No sporangia of the widely distributed *Onychiopsis* have been described;[1] that generic name was substituted for *Sphenopteris* because of the discovery of fertile pinnae in Japan, England, and elsewhere similar to those of the Polypodiaceous genus *Onychium*. It is not easy, in the absence of fertile specimens, to distinguish between pinnae of *Onychiopsis* and *Ruffordia*, and the polymorphism of *R. Goepperti* adds to the difficulty. Berry,[2] in describing different forms of sterile fronds from the Potomac formation of eastern North America, includes in *Onychiopsis* both *O. psilotoides* and *O. Goepperti*: as fertile specimens were not available *Sphenopteris* would have been the more appropriate designation: there is no justification for the substitution of *Onychiopsis* for *Ruffordia*. The name *Ruffordia*[3] was first suggested in place of *Sphenopteris* for *S. Goepperti*, because it was believed that the habit of sterile and fertile pieces of fronds indicated affinity to species of *Aneimia*. Spores of the Schizaeaceous type were subsequently obtained from English material,[4] and more satisfactory confirmation was supplied by Halle,[5] who found numerous impressions of apical annuli associated with pinnae of *Ruffordia Goepperti* in beds, presumably of Lower Cretaceous age, in Manchuria. It is noteworthy that the fronds of *Ruffordia Goepperti* agree very closely in habit with those of some recent species of *Aneimia*, e.g. *A. adiantifolia*, one of the solenostelic species already mentioned. The leaves of recent species of *Aneimia* are crowded, and leave no free surface of stem between them, a feature shared with *Tempskya*. Moreover, the occurrence of several small cells at the distal end of the annulus (Fig. 8) is a feature consistent with affinity to *Aneimia* rather than to *Schizaea* or *Lygodium*, in the sporangia of which there is a much smaller distal region.[6] Fronds agreeing closely in habit with *Ruffordia Goepperti*, or identical with that species, occur in the Kootanie formation of North America, in the Wealden of England, and in the Lower Cretaceous of Bohemia, in association with *Tempskya*.

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[1] Gwynne-Vaughan (1903).

[2] Thompson (1918).

[3] Boodle (1895).

[4] Schoute (1906).

[5] Bommer (1910). See also Seward (1922), p. 230.

[6] Seward (1913), Plate XIII.

[7] Zeiller (1914).

[8] Seward (1910), p. 348.

Two other Wealden Ferns should be mentioned. *Schizaeopsis expansa* (Font.), described by Berry,[7] from the lowermost Cretaceous beds of the Atlantic Coastal Plain, has fronds of the *Schizaea* type, and spores very like those described by Boodle in the false stem of *Tempskya erosa*. It is, however, unlikely that this species represents the foliage of *Tempskya*, as recent species of *Schizaea* all differ anatomically from *Tempskya* stems. Berry points out that sterile fronds very similar to *Schizaeopsis* occur in the Kootanie formation, the home probably of *Tempskya Knowltoni*; but the geographical range of *Ruffordia* is wider, and on other grounds that genus is more closely connected with *Tempskya*. The species *Pelletieria valdensis*,[1] founded on portions of fertile fronds and well-preserved spores very similar to those described by Boodle, is probably another example of a Wealden Schizaeaceous Fern.

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[1] Seward (1910), p. 378; (1913), p. 96. For a fuller account see Seward (1894), p. 40.

[2] Berry (1911), p. 267.

[3] Seward (1894), p. 75.

[4] Seward (1913), p. 91.

[5] Halle (1921), Plate II, Figs. 1–8.

[6] Bower (1923), p. 253.

[7] Berry (1911^{2}).

Its affinity to recent members of the Schizaeaceae has not been thoroughly established, but it may afford another example of the wide range in form and wealth of species characteristic of this family in the later Mesozoic epoch in the Northern Hemisphere.[2] The contrast between the past and present distribution of the Schizaeaceae has been dealt with elsewhere.

An Upper Cretaceous Japanese Fern stem, described by Miss Kershaw[3] as *Solenostelopteris japonica*, agrees with *Tempskya* in the exarch position of the narrowest tracheides, but the resemblance is not otherwise very close, and the roots are of a different type.

CONCLUSION AND SUMMARY.

The specimen on which the new species *Tempskya Knowltoni* is founded was obtained from Montana, from beds in all probability belonging to the Lower Cretaceous, Kootanie formation. It agrees generally in the structure of the stems, petioles, and roots with *T. rossica* and with other species of the genus. The false stem consists of a mass of diarch, branching roots enclosing slender, solenostelic stems bearing, usually on the same side of the axis, two series of alternate, contiguous leaves. The stems, which increase in number from the narrow end of the specimen upwards, show frequent dichotomous branching. The xylem of the stele has no protoxylem groups within the metaxylem and little or no xylem parenchyma; the leaf-traces arise as loops of the solenostele, and pass out alternately right and left to the petioles. A piece of a root, believed to be Araucarian, was found among the matted roots of the Fern, also a few groups of thick-walled cells which may be apical annuli of Schizaeaceous sporangia.

It is impossible, on the information available, to assign the recorded examples of *Tempskya* to species distinguished by clearly defined characters. The only specimens sufficiently well preserved to furnish data for a fairly complete anatomical description are the type-specimen of *Tempskya rossica* and the Montana fossil.

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[1] Seward (1913), p. 91.

[2] Seward (1922), Map C. I take this opportunity of correcting a careless mistake on p. 222 of the Hooker Lecture (1922): leaves of a fossil species of *Onoclea* are there stated to be characteristic of Upper Cretaceous beds in the United States. I am indebted to Dr. Hollick and Dr. Knowlton for calling my attention to the fact that the beds are Eocene in age. This correction has an important bearing on the remarks made on p. 223 of the Lecture.

[3] Kershaw (1910).

The English specimens frequently referred to *T. Schimperi*, one of the species founded by Corda, should, as Dr. Marie Stopes[1] pointed out, be named *T. erosa*, as that is the older specific name. As previously shown, it is hopeless to attempt to discriminate between the species described by Corda. There are no satisfactory grounds for drawing a distinction between the English specimens and those from Germany, Bohemia, and France, but there is something to be said in favour of retaining the name *T. Schimperi*, as Dr. Stopes suggested, for the European continental species, confining the designation *T. erosa* to the English examples. All the specimens of *Tempskya* so far discovered may belong to one species; the retention of different specific names may be defended on the ground of convenience and as a provisional course justified by lack of knowledge. The important point is that the various forms of the genus conform to the same anatomical type: dichotomously branching stems with a solenostelic vascular system, meristeles of the horseshoe form, and diarch roots.

Tempskya erosa (Stokes and Webb, and Mantell). Characteristic of English Wealden beds, and represented in the Lower Greensand. The stems are rather larger in diameter (Fig. 4, Pl. XVI; Fig. 16, Pl. XVII) than those of *T. Knowltoni.*

Tempskya Schimperi, Corda. Perucer formation (Lower Cretaceous) of Bohemia; Wealden of Germany and France. Probably specifically identical with *T. erosa*; stems rather stouter than in *T. Knowltoni.*

Tempskya Whitei, Berry. Patapsco formation (Lower Cretaceous) of Maryland. Considered by Mr. Berry to be distinct from the European species, but not fully described.

Tempskya rossica, Kidston and Gwynne-Vaughan. Tertiary conglomerate, Mougadjar Mountains, Russia: [probably derived from Cretaceous rocks].

Tempskya Knowltoni, sp. nov. Probably from the Kootanie formation (Lower Cretaceous), Montana.

The wide geographical distribution of this well-defined type of Fern furnishes an additional piece of evidence consistent with the opinion, based on a comparison of Wealden floras, that in the early part of the Cretaceous period climatic conditions were more uniform than they are at the present day.

In comparison with specimens of the genus recorded from other localities, especially many of those from English rocks, the American fossil is relatively small. The restoration by Kidston and Gwynne-Vaughan represents the false stem of *Tempskya rossica* as a thick, vertical column, the erect position being inferred from lack of any constant orientation of leaves in relation to stems and from the vertical course of the roots. In *T. Knowltoni* the position of the leaves is much less inconstant. The fact that the roots pursue in the main a vertical course is not proof of an erect position: hydrotropism as well as geotropism must be taken into account, and an examination of recent Ferns shows that the direction of the roots is not a trustworthy criterion of the position of the stem. The occurrence of two well-preserved foreign roots (probably Araucarian) among the matted roots of *Tempskya* is regarded as evidence that the Fern was below the surface of the ground. It does not necessarily follow that the Conifer roots penetrated the tissues of *Tempskya* during the life of the Fern, but the state of preservation of the intruded roots and those of *Tempskya* suggests that they were associated when alive.

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[1] Stopes (1915), p. 18.

The published restoration of *Tempskya rossica*, like many attempts to picture the habit of plants never seen alive by any human eye, creates an impression of unreality. I prefer to think of *T. Knowltoni* as a root-encircled bundle of stems, obconical and tapering, lying obliquely in the soil, a few of the stem branches bearing crowded fronds near the ground-level.

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EXPLANATION OF PLATES XVI AND XVII.

Illustrating Prof. A. C. Seward's paper on a New Species *of Tempskya* from Montana.

(With the exception of Figs. 1 and 16 the photographs are by Mr. Tams.)

PLATE XVI.

Fig. 1. The specimen of *Tempskya Knowltoni* as it was found. $\frac{7}{10}$ nat. size.

Fig. 2. Longitudinal section of part of a stem showing a group of dark oval bodies in the inner cortex, *i.c.* × 26. Section x.

Fig. 3. The apex of the false stem. S., one of the stems. Nat. size.

Fig. 4. Transverse section of *Tempskya erosa*. P., petiole attached to a stem. British Museum coll. Locality unknown. (V. 8214.)

Fig. 5. Apical annulus of a sporangium, probably of *Tempskya Knowltoni.* × 85. Section vii.

Fig. 6. Transverse section of two meristeles, *m.*, enclosed in a common cortex. × 12. Section ii.

Fig. 7. Transverse section of a root showing the outer cortex, *o.c.* × 45. Section v.

Fig. 8. Apical annulus, enclosing a group of small cells. × 85. Section vii.

Fig. 9. Complete transverse section cut at the level vii, Text-fig. 1, showing several stems among the roots. × 2. Section vii.

Fig. 10. Enlarged photograph of the branching stem near the centre of Fig. 9 (stem 14, Text-fig. 2). × 11. Section vii. *a*, outer part of the central sclerenchyma.

Fig. 11. Longitudinal section of a stem showing two petioles, P., P′. S., pad of sclerenchyma at the base of the lower petiole. × 7. Section xi.

Fig. 12. Transverse section of the stem shown in Fig. 10 after the separation of the branches. R., root. × 11. Section viii.

Fig. 13. Longitudinal section of a stem. P., P., P., petioles; *i.c.*, inner cortex. × 7. Section xiv.

PLATE XVII.

Fig. 14. Transverse section of a petiole base. × 28. Section iv.

Fig. 15. Longitudinal section of the inner sclerenchymatous cortex of a root showing the xylem of a lateral branch. × 50. Section xii.

Fig. 16. Transverse section of the stele of a stem of *Tempskya erosa* with two meristeles at the upper end. From the Imperial College coll. × 15. Phot. Dr. Kidston.

Fig. 17 Roots in transverse section. *o.c.*, outer cortex.

Fig. 18. Branching root showing the endodermis, *e.* × 35. Section vii.

Fig. 19. Transverse section of a root with outer cortex, *o.c.* *e.*, endodermis. × 50. Section vii.

Fig. 20. Part of the stele of a stem. *a*, brown deposit at the outer edge of central sclerenchyma; *i.c.*, inner cortex. × 70. Section v.

Fig. 21. Transverse section of a Gymnospermous root, probably Araucarian. P., periderm. × 35. Section vii.

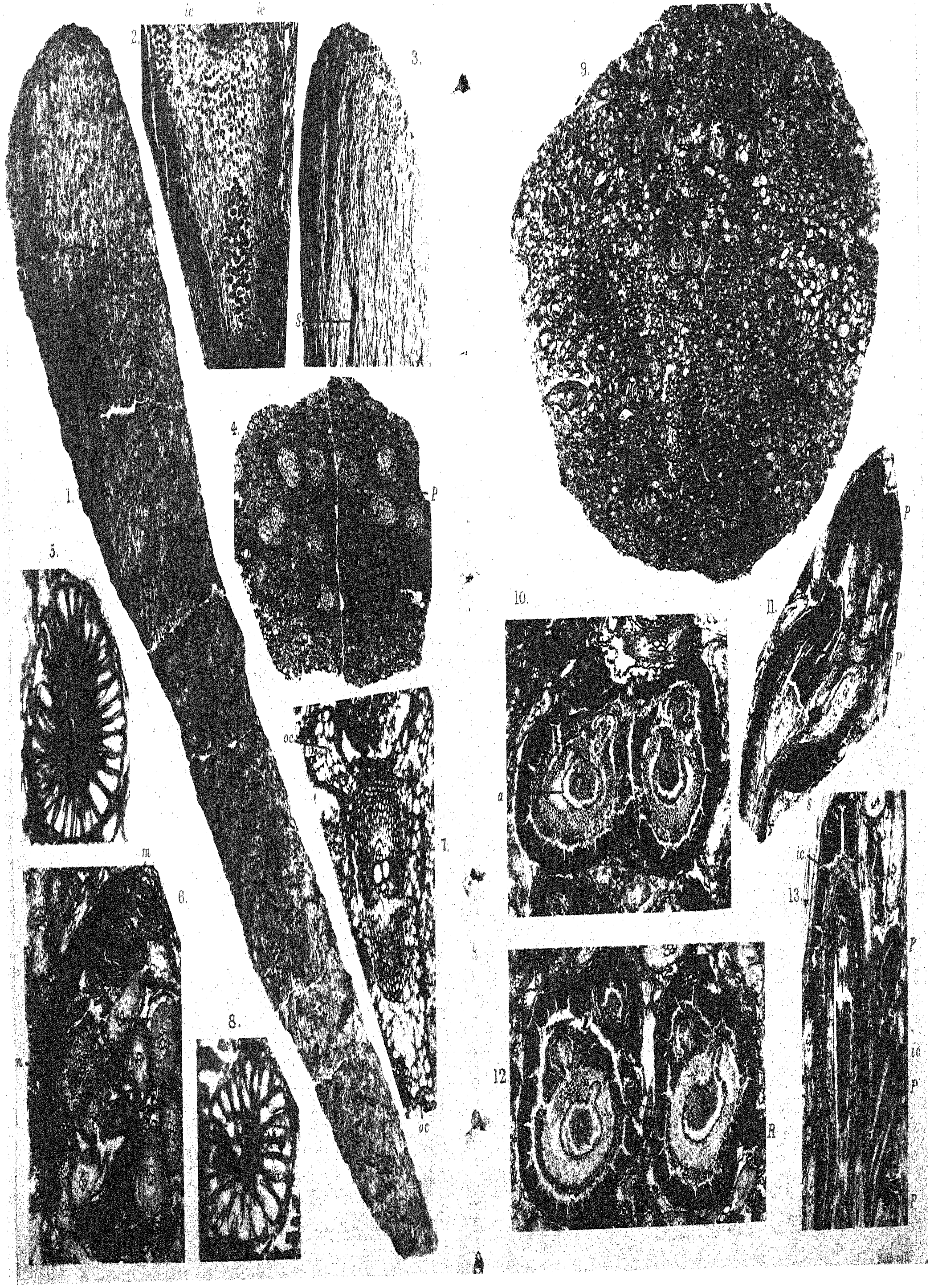
Fig. 22. Part of the xylem of the Gymnospermous root enlarged, showing the alternate, contiguous pits on the tracheides. Section xvi.

Fig. 23. Transverse section of the central region of a young root. *e.*, endodermis. Section viii.

Fig. 24. Branching root, showing dark, oval bodies in the central region. × 11. Section vii.

Fig. 25. Transverse section of stem 23, Text-fig. 2, showing many invading roots. × 11. Section vii.

Fig. 26. Transverse section of a petiole base attached to a stem. PX., protoxylem. × 40. Section ix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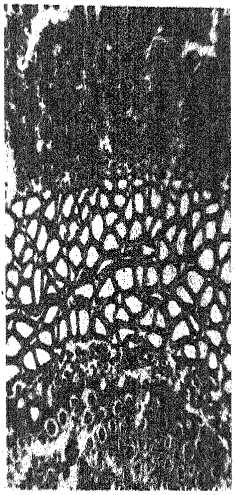

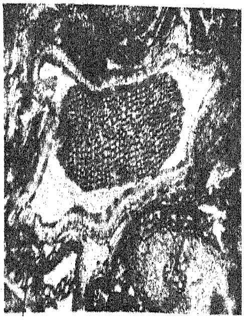

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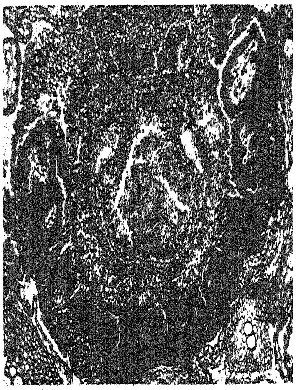

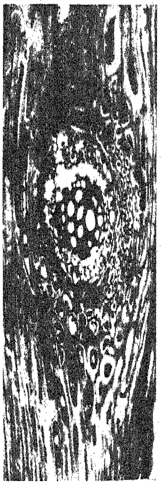

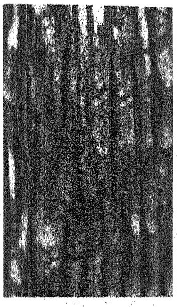

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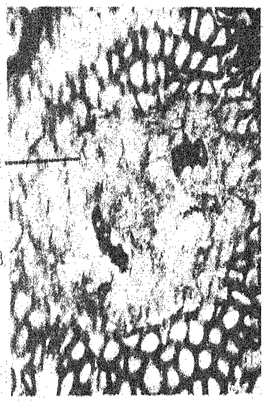

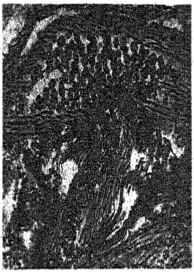

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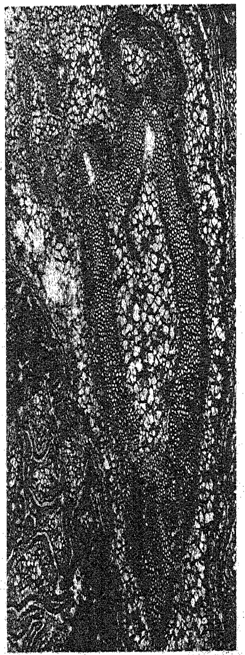

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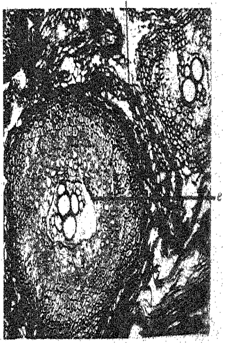

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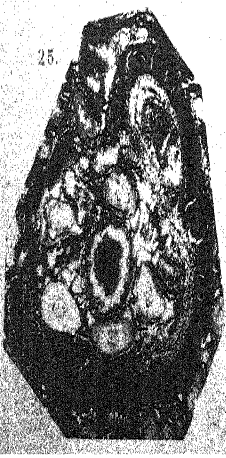

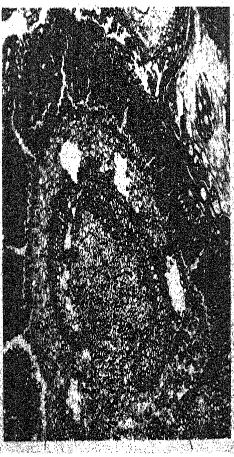

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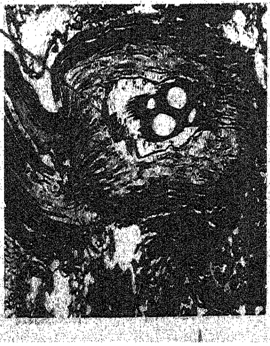

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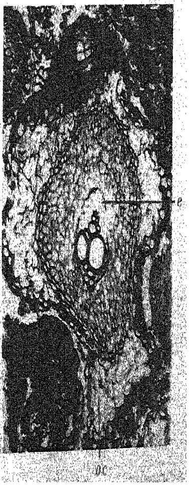

Duration of Light and Growth.

BY

J. ADAMS.

DURING the last few years considerable attention has been devoted to the effect of light, as regards its duration, apart from its intensity, on plant-growth. In some cases the normal period of daylight at the time of the year when the experiments were made has been shortened by the use of dark coverings or dark chambers, while in other cases the period of illumination has been lengthened by the use of electric light. While electric light is probably not quite the same in its effects as natural daylight of similar intensity, still it is the only practicable method of lengthening the light period beyond a daily average of 16 hours in latitudes south of 46° in the Northern Hemisphere. That it forms an efficient substitute for natural daylight is shown by the observations of Pendred (4) on the moss-like vegetation growing near electric lamps inside Cheddar caves. Steel (6) refers to observations made by Cheel at Sydney, Australia, in 1912, where plane trees growing near large electric lamps retained their leaves a month longer on those branches nearest to the electric light. I have observed the same result in the case of Norway maple at the Experimental Farm, Ottawa, but whether the branches nearest to the electric lamp retained their leaves longer owing to the heat from the lamp or the illumination at night is not at present clear. However, any doubt as to the value of electric light in promoting plant-growth has been set at rest by the experiments of Harvey (3) at St. Paul, Minnesota, who grew a great variety of plants, including cereals, buckwheat, peas, lettuce, &c., to maturity under electrical illumination alone, daylight having been entirely excluded.

In a former paper (1) I gave some account of the effect of shortening the natural length of day on various plants as regards height, weight, and time of flowering. The object of the present investigation was to experiment with a larger range of light exposures, including electrical illumination, in order to determine whether there was an upper limit to the daily number of

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hours of light which a plant could usefully employ. The daily exposures to light in these experiments varied from 3 hours up through various intermediates to 20 hours.

While annual plants were used in most of the experiments, in one case observations were made on Onion bulbs and in another case on Potato tubers.

The plants were grown in a greenhouse and all the experiments were done in duplicate or triplicate, mostly the latter. The number of plants experimented with in each case varied, but they were sufficiently numerous to give a fair average result. All the other conditions, such as moisture, temperature, soil, &c., were kept as uniform as possible, the light factor being the only one that varied.

EXPERIMENTS WITH ONION BULBS, 1922.

Small Onion bulbs were planted in pots on 6 March 1922, one in each pot. One set was subject to the alternation of day and night, while the other was covered by a wooden box and kept continuously in the dark.

On 27 March the average height of the set exposed to light was 11 mm., while that of the darkened set was 34 mm.

In order to keep the darkened plants alive and healthy the wooden box was subsequently removed. It was found on 18 April that the average height of the set constantly exposed to light was 276·0 mm., while that of the set previously darkened was 252·3 mm.

EXPERIMENTS WITH POTATO TUBERS, 1922.

Six small tubers were planted on 11 March 1922, one in each pot. Three were exposed to day and night conditions in the greenhouse, while the other three were placed beside them and covered with a wooden box to exclude the light.

Of the plants exposed to light two sprouts were just breaking through the surface of the soil on 10 April, one in each pot, while in the third pot no growth was yet visible. On the same date in the darkened pots the average height of the shoots was 18·3 mm. On 18 April the average height of the shoots in the plants exposed to light was 23·3 mm., while in those kept in the dark the average height was 75·6 mm.

EXPERIMENTS WITH WHITE MUSTARD, 1922.

Series I.

Seeds were planted in pots on 25 April 1922, one set being exposed to light and the other kept dark.

The average height of the plants kept dark was 87·7 mm. on 4 May, while that of the plants exposed to light was 19·7 mm.

As it was not possible to keep the darkened plants in a healthy condition much longer, another series of experiments was started.

Series II.

In this experiment, instead of keeping one set of plants in complete darkness, they were exposed to light at intervals, while the other set was exposed to the regular alternation of day and night. The seeds in both pots were sown on 5 May 1922. The covering was removed from the darkened set on various dates from 9 May to 26 May, so that the total amount of exposure to light between these dates was 60 hours, and the time of exposure on any one day varied between 2 and 7 hours. On 27 May the covering was removed and was not again replaced.

The average height of the plants in the darkened set on 9 May was 19·9 mm., while on the same date the average height of the exposed set was 6·4 mm.

On 2 June the average heights of the darkened and fully exposed sets were 87·6 and 100·6 mm. respectively.

EXPERIMENTS WITH FLAX, 1922.

Series I.

Seeds were planted in pots on 25 April 1922, one set being exposed to light and the other kept dark.

The average height of the plants kept dark was 71·6 mm. on 5 May, while the average height of the plants exposed to light was 16·2 mm. on the same date.

Series II.

As in the case of White Mustard, one of the two sets of Flax plants was uncovered at intervals, while the other was left uncovered. The covering was removed from the darkened set on various dates from 11 May to 23 May, so that the total amount of exposure to light between these dates was 47 hours, and the time of exposure on any one day varied between 3 and 7 hours. The seeds were sown on 5 May 1922.

The average height of the plants in the darkened set was 19·2 mm. on 11 May, while the average height of the exposed set on the same date was 7·1 mm. The respective heights of the darkened and exposed sets on 26 May were 68·5 and 90·2 mm.

EXPERIMENTS WITH WAX BEAN, 1922.

In order to test the behaviour of larger seeds under similar conditions to those previously mentioned, an experiment was made with seeds of Wax Bean and Sunflower.

Seeds of Wax Bean were planted on 27 May 1922. One set was exposed to the light during the course of the experiment, while the other was uncovered on various dates from 2 June to 14 June. On 15 June the cover was removed and was not again replaced. The total amount of exposure between these dates was 40¼ hours, and the time of covering on any one day varied between 2½ and 6¼ hours.

The average height of the plants in the darkened set was 140·3 mm. on 5 June, while on the same date the average height of the exposed set was 83·4 mm.

The average height of the plants in the set previously covered was 424·1 mm. on 6 July, while on the same date the average height of the exposed set was 275·8 mm.

The first flower in the darkened set opened on 6 July, while in the exposed plants the first flowers opened on 5 July.

EXPERIMENTS WITH SUNFLOWER, 1922.

Seeds were planted on 27 May 1922. The period of exposure to light in the two sets was the same as in the case of Wax Bean.

The average height of the plants in the darkened set was 30·3 mm. on 2 June, while on the same date the average height of the exposed plants was 11·6 mm. The average height of the plants in the darkened set was 261·1 mm. on 27 June, while on the same date the average height of the exposed set was 304·5 mm.

EXPERIMENTS WITH ELECTRICAL ILLUMINATION, 1922.

In order to test the effect of supplementing the ordinary period of daylight with artificial illumination at night, experiments were undertaken with Winter Wheat, Winter Rye, Flax, White Mustard, and Shamrock (*Trifolium dubium*, Sibth.). At first a 100-watt nitrogen-filled lamp was used; subsequently this was replaced by a 200-watt lamp, and finally a double socket was employed, carrying both a 200-watt and a 100-watt lamp. The plants were illuminated as a general rule on five nights in each week (Monday to Friday inclusive). The lights were turned on at dusk and left burning until about 7.30 on the following morning. The plants exposed to daylight only were screened from the effects of the electric light by a framework covered with brown paper.

KHARKOV WINTER WHEAT, 1922.

Seeds were planted on 31 October 1922. Illumination with an electric lamp commenced on 11 December 1922, continued until 23 March 1923, and occurred on 67 nights.

The average height of the daylight set of plants (measured to the tip of the leaf) was 122·2 mm. on 11 December, while the average height of the illuminated set on the same date was also 122·2 mm.

On 28 March 1923 the average heights (measured to the top of the uppermost sheath) of the daylight and illuminated sets were 146·9 mm. and 500·6 mm. respectively.

In the daylight set of plants the first plant came into ear on 2 May 1923, while in the illuminated set the first plants came into ear on 5 April 1923.

COMMON WINTER RYE, 1922.

The particulars regarding illumination were the same as for Winter Wheat. The seeds were sown on 31 October 1922. The average height of the daylight set of plants (measured to the tip of the leaf) was 102·7 mm. on 11 December, while the average height of the illuminated set on the same date was 100·4 mm.

On 27 March 1923 the average height (measured to the top of the uppermost leaf-sheath) was 148·0 mm. in the case of the daylight plants, while in the illuminated plants the corresponding figure was 839·4 mm.

In the daylight set the first appearance of the ear protruding from the enclosing sheath was on 14 April 1923, while in the illuminated set the date was 28 February 1923.

FLAX, 1922.

One set was exposed to the alternation of day and night, while the other was also illuminated at night. The period of electrical illumination extended from 18 December 1922 to 23 March 1923, and included 62 nights.

The seeds were sown on 24 November 1922. The average height of the daylight and illuminated sets on 18 December was 27·2 mm. and 26·8 mm. respectively.

On 26 March the average heights were 322·9 mm. for the daylight plants and 344·2 mm. for the illuminated plants.

On 23 April there were in flower 12 plants in the case of the daylight set, while in the illuminated set 16 were in flower on the same date.

WHITE MUSTARD, 1922.

One set was exposed to the alternation of day and night, while the other was in addition illuminated at night. The period of electrical illumination extended from 12 December 1922 to 23 March 1923, and included 66 nights.

The seeds were sown on 24 November 1922. The average height of the daylight and illuminated sets on 12 December was 45·1 mm. and 42·8 mm. respectively.

On 7 April 1923 the average heights of the daylight and illuminated sets were 761·6 mm. and 1012·0 mm.

In the plants exposed to daylight only two plants had come into flower on 7 April 1923, while in the illuminated set on the same date there were 15 plants in flower.

SHAMROCK (*TRIFOLIUM DUBIUM*, Sibth.), 1922.

For this experiment small pots, each containing a single plant, were used, one set being employed for the daylight experiment, while the other set was employed for daylight plus artificial illumination. The plants were of approximately equal size when the experiment commenced on 12 December 1922, and the electrical illumination was continued to 23 March 1923, and included 66 nights.

On 26 March the average heights in the daylight and illuminated plants were 304·0 mm. and 448·5 mm. respectively.

In the plants exposed to daylight only the first date of coming into flower was on 12 April, while in the illuminated plants the date of first flowering was on 31 March.

EXPERIMENTS WITH ELECTRIC LIGHT, 1923.

In order to test still further the relation of growth to varying amounts of light, experiments were devised in which the plants were exposed to light for 3, 6, 12, and 18 hours daily during the earlier months of spring. Later on, when the days had become longer, another set of plants was employed with exposures of 5, 10, 15, and 20 hours.

For the purpose of exposing the plants to light for periods of 3, 5, 6, and 10 hours, box-like coverings were made which were put over the plants in the greenhouse or removed as required. These coverings were made of light wooden laths nailed together and covered on the bottom, sides, and ends with sheets of brown paper. A space of about 3 in. all round the top was left uncovered, so as to admit air between the pots when the cover was inverted and placed in position. At the same time very little light was admitted, as the pots used were about 8½ in. high. These coverings measured 3 ft. 3 in. long, 2 ft. 3in. wide, and 2 ft. 6 in. deep.

The exposures for 12 and 15 hours were obtained by leaving the pots entirely uncovered at the time of the year when the average length of day was of this duration.

The exposures for 18 and 20 hours respectively were obtained by the use of nitrogen-filled lamps during certain nights each week to supplement the regular period of daylight. A double socket was used carrying lamps of 200 watts and 100 watts capacity for part of the time, and for the remainder two lamps each of 200 watts on a voltage of 110. The plants nearest to the light were at a distance of 42 in. and those farthest away were at a distance of 60 in. The total period of electrical illumination extended from 26 March to 28 June.

In all the species under consideration the heights of the plants were measured at intervals of about a week, but only the first and last measurements as a rule are given, as these show the greatest contrast. The measurements are mentioned in the same order as the periods of illumination, the first figures referring to the shortest period of exposure to light.

SPRING WHEAT, 1923.

The variety used was Marquis. There were four sets to be exposed to light daily for 3, 6, 12, and 18 hours respectively. The seeds were sown on 21 March 1923. Electrical illumination occurred on 36 nights.

On 3 April the average height (measured to the tip of the leaf) was in the four sets 84·6 mm., 71·0 mm., 65·3 mm., and 65·0 mm.

On May 11 the heights (measured to the top of the uppermost sheath) were 58·1 mm., 122·5 mm., 224·9 mm., and 319·1 mm.

The first plants under the 12-hour exposure came into ear on 30 May; this set produced a total of 35 ears and weighed when cut on 7 June 224·530 grm.; the corresponding figures for the 18-hour set were 22 May, 43 ears, and 233·690 grm.

ALSIKE CLOVER, 1923.

There were four sets arranged in groups for exposure to light for 3, 6, 12, and 18 hours respectively.

The seeds were sown on 21 March 1923. Electrical illumination occurred on 18 nights. On 4 April the average heights of the plants in each set were 6·5, 5·0, 2·7, and 1·4 mm.

As the plants grew very slowly and developed leaves at the ground level instead of elongated shoots, they were cut off on 7 May and weighed. The average weight (fresh) of the plants successively exposedto longer periods of light was 0·012, 0·054, 0·212, and 0·664 grm.

FLAX, 1923.

There were 4 sets for exposure to light of 3, 6, 12, and 18 hours' duration respectively. The seeds were sown on 21 March 1923. Electrical illumination occurred on 19 nights. On 3 April the average heights were 43·2 mm., 32·3 mm., 21·7 mm., and 18·8 mm.

The final measurements on 8 May gave 128·1 mm., 194·3 mm., 290·1 mm., and 295·4 mm.

BUCKWHEAT, 1923.

There were 4 sets arranged in groups for exposure to light for 3, 6, 12, and 18 hours respectively. The seeds were sown on 21 March 1923. Electrical illumination occurred on 19 nights.

The average heights on 5 April were 42·6 mm., 25·5 mm., 18·1 mm., and 19·7 mm.

On 14 April the heights were 72·6 mm., 67·1 mm., 58·0 mm., and 51·9 mm.

The final measurements on 8 May gave 131·7 mm., 260·3 mm., 575·1 mm., and 525·3 mm.

The plants were cut at the ground level on 8 May and weighed. The total weights (fresh) were 4·550 grm., 16·770 grm., 120·250 grm., and 187·770 grm.

With regard to the production of flowers, on 8 May 7 plants were in flower in the 12-hour set, while only 2 were in flower in the 18-hour set. No flowers had opened on this date in the other 2 sets.

TOMATO, 1923.

There were 4 sets which were exposed to light for periods of 3, 6, 12, and 18 hours respectively, up to 12 May, but after that date the times of exposure were 5, 10, 15, and 20 hours. Electrical illumination occurred on 37 nights.

The seeds were sown on 21 March 1923. The average heights on 17 April were 28·7 mm., 26·1 mm., 17·3 mm., and 14·5 mm.

The final measurements on 29 May gave 64·5 mm., 191·8 mm., 369·0 mm., and 373·1 mm.

All the plants were cut at the ground level on 8 June and weighed. The total weights were 7·530 grm., 136·140 grm., 613·720 grm., and 734·050 grm.

With respect to the development of flowers, the 12-hour and 18-hour sets came into flower on the same date, while in the other 2 sets none had come into flower on 8 June.

SOY BEAN, 1923.

There were 4 sets for exposure to light for 3, 6, 12, and 18 hours. After 12 May the exposures were for 5, 10, 15, and 20 hours. Electrical illumination occurred on 36 nights.

The seeds were sown on 24 April 1923. On 11 May the average heights were 16·6 mm., 17·9 mm., 22·3 mm., and 17·0 mm.

On 30 May the average heights were 160·7 mm., 150·4 mm., 110·0 mm., and 103·3 mm.

The final measurements on 6 July were 418·2 mm., 938·3 mm., 1156·5 mm., and 1150·0 mm.

The plants were cut at the ground level on the above date and weighed. The total weights of each set were 37·040 grm., 64·910 grm., 159·310 grm., and 180·080 grm.

At the end of the experiment all the plants had developed a twining habit. In the set exposed longest to daylight the first flowers opened on 22 June, while in the set under electrical illumination no flowers were produced up to 6 July, when the experiment ended.

KHARKOV WINTER WHEAT, 1923.

There were 2 sets for exposure to light for 12 and 18 hours respectively. After 12 May the exposures were for 15 and 20 hours. Electrical illumination occurred on 49 nights.

The seeds were sown on 21 March 1923. The average heights on 4 April (measured to the tip of the leaf) were 71·7 mm. and 64·1 mm.

The last measurements were made on 12 May. The average heights to the top of the uppermost leaf-sheaf were 83·6 mm. and 86·8 mm.

In the daylight set none were in ear on 12 July, while on 12 September 2 plants were in ear, and there were 3 ears altogether. In the electrically illuminated set 2 plants were in ear on 12 July, while on 12 September 7 were in ear, and there were 15 ears altogether.

WINTER RYE, 1923.

There were 2 sets for exposure to light for 12 and 18 hours respectively. After 12 May the exposures were for 15 and 20 hours. Electrical illumination occurred on 49 nights.

The seeds were sown on 21 March 1923. The average heights on 4 April (measured to the tip of the leaf) were 82·6 mm., 66·8 mm.

The last measurements were made on 12 May. The average heights to the top of the uppermost leaf-sheath were 101·0 mm. and 112·3 mm.

On 11 June the first plant in the daylight set came into ear, while in the illuminated set the first plant came into ear on 28 May.

On 12 September in the daylight set 5 plants were in ear, and there were 29 ears altogether, while in the electrically illuminated set there were 6 plants in ear, and a total of 48 ears altogether was produced.

CASTOR-OIL PLANT, 1923.

There were 4 sets for exposure to light for 5, 10, 15, and 20 hours respectively. The seeds were sown on 8 May, 1923. Electrical illumination occurred on 30 nights.

On 5 June the average heights were 126·8 mm., 126·8 mm., 107·0 mm., and 141·5 mm.

The final measurements on 11 July gave 290·6 mm., 343·1 mm., 472·0 mm., and 462·3 mm. The plants were cut at the ground level on this date and weighed. The total weights (fresh) were 103·890 grm., 121·160 grm., 200·990 grm., and 219·160 grm.

SUNFLOWER, 1923.

There were 4 sets for exposure to light for 5, 10, 15, and 20 hours respectively. The seeds were sown on 9 May 1923. Electrical illumination occurred on 29 nights.

The average heights on 21 May were 65·4 mm., 39·6 mm., 31·6 mm., and 39·6 mm.

On 12 June the averages were 241·7 mm., 281·3 mm., 349·6 mm., and 415·0 mm.

The final measurements of the 15-hour and 20-hour sets on 5 July gave 888·7 mm. and 949·0 mm. The plants were cut off at the ground level on this date. The total weights (fresh) were 310·890 grm. and 336·790 grm.

HEMP, 1923.

There were four sets which were exposed to light for 5, 10, 15, and 20 hours. The seeds were sown on 9 May 1923. Electrical illumination occurred on 29 nights.

On 25 May the average heights were 91·8 mm., 88·9 mm., 74·2 mm., and 63·2 mm. respectively.

The average heights on 13 June were 138·6 mm., 161·5 mm., 443·5 mm., and 389·2 mm.

The final measurements on 9 July of the 15-hour and 20-hour sets gave 935·5 mm. and 788·5 mm.

On 12 July the plants were cut at the ground level and weighed. The total weights (fresh) of the above 2 sets were 216·540 grm. and 178·680 grm.

FLAX, SECOND SERIES, 1923.

There were 4 sets which were exposed to light for 5, 10, 15, and 20 hours respectively. The seeds were sown on 11 May 1923. Electrical illumination occurred on 29 nights.

On 26 May the average heights were 80·8 mm., 76·3 mm., 67·2 mm., and 70·2 mm.

The average heights on 11 June were 142·1 mm., 185·7 mm., 242·0 mm., and 246·8 mm.

The first plants in the 15-hour set came into flower on 6 July, and on 7 July 5 were in flower. In the 20-hour set the first plant came into flower on 25 June, and on 7 July 13 were in flower.

The plants in these two sets were pulled up by the roots on 7 July and weighed. The total weights (fresh) were 78·790 grm. and 72·490 grm.

BUCKWHEAT, SECOND SERIES, 1923.

There were four sets which were exposed to light for 5, 10, 15, and 20 hours respectively. The seed was sown on 11 May 1923. Electrical illumination occurred on 21 nights.

On 21 May the average heights were 61·5 mm., 42·4 mm., 29·0 mm., and 38·2 mm.

The final measurements on 11 June gave 176·9 mm., 187·5 mm., 384·4 mm., and 397·1 mm.

On 18 June the plants were cut off at the ground level and weighed. The weights (fresh) of the 15-hour and 20-hour sets were 249·440 grm. and 243·580 grm.

On 18 June 9 plants were in flower in the 15-hour set and 8 plants in the 20-hour set, but no flowers had yet opened in the other two.

DISCUSSION OF RESULTS.

The results of the experiments referred to on the previous pages may conveniently be grouped under three divisions:

A. Experiments with daylight only in 1922.

B. Experiments with daylight supplemented by electrical illumination in 1922 and 1923.

C. Experiments with three series of exposure to daylight and one with daylight plus electrical illumination in 1923.

In group *A* the results obtained in the case of Onion, Potato, White Mustard, Flax, and Sunflower were uniform, namely, growth was greatest at first in the darkened plants, but at the end of the experiment the illuminated plants were the tallest.

In the case of Wax Bean, however, the darkened plants were the tallest at first and also at the end of the experiment.

In group *B* those exposed to daylight were at first equal to or taller than those exposed to electrical illumination, but in the end the latter were both taller and flowered earlier. In group *B* were included Winter Wheat, Winter Rye, Flax, White Mustard, and Shamrock.

In group *C* the results were much more varied, but in all of the species except Castor-oil Plant those exposed to light for the shortest period were the tallest at first. In this group were included Spring Wheat, Alsike Clover, Buckwheat, Tomato, Soy Bean, Sunflower, and Hemp.

Until further experiments have been made it is scarcely possible at present to say what was the actual effect of the electrical illumination in every case. During the shorter days of winter it appears to have exercised a beneficial effect in hastening growth and time of flowering. But when the natural length of day varied between 12 and 15 hours the addition of artificial illumination produced little result. The most remarkable effect was in the case of Soy Bean, where flowers were completely absent in each of the three pots exposed to electric light.

In the case of the plants exposed to daylight only for varying lengths of time the results were quite uniform. The plants receiving daylight for 3 or 5 hours were the tallest at first, those exposed for 12 to 15 hours were the shortest at first, and those receiving 6 to 10 hours occupied an intermediate position. At the end of the experiment the positions were reversed, those receiving most light being the tallest. It follows, therefore, that where plants are equally supplied with reserve material, growth—that is, extension in length of an axial organ—takes place more rapidly in darkness or diminished light. In the end, however, those plants exposed longest to the action of light, being enabled to accumulate a larger supply of reserve material for the formation of new tissue as the result of photosynthesis, ultimately attained the greatest height.

The Wax Bean behaved at first like the other species, but whether the final result was different owing to the large amount of reserve material in the seed it would scarcely be possible to say without further experiment.

On comparing the above results with those obtained by other experimenters there appears to be very considerable difference of opinion as to the action of light on plant-growth. Garner and Allard, in a second paper (2), give a detailed account of an extensive series of experiments with about 55 different species of plants, including cereals, weeds, bulbous and tuberous plants, marsh plants, garden flowers, and various trees and shrubs. They experimented not only with various periods of daylight, but also with electrical illumination in addition. Their conclusions deal with the relation of length of day to the initiation and regulation of sexual reproduction in plants, the formation of tubers and bulbs, the character and extent of branching, root growth, formation of pigment, abscission and leaf-fall, dormancy, and rejuvenescence. 'The evidence available at this time indicates that for each species there is an optimal light period for maximum upward elongation of the stem or increase in height. For some species this optimal light period is furnished by the longest days of summer in the temperate zone, while for other species the intermediate length of day of spring and fall is optimal. In the first-named group of species progressive shortening of the daily light period initiates a series of responses, including flowering and fruiting, tuberization, character and extent of branching, dormancy, &c. With day lengths ranging between the optimal for growth and the optimal for sexual reproduction the tendency towards division of the plant's energies between these two types of activity is manifested in the ever-blooming or ever-bearing condition, change in the size of the individual fruit or seed, cleistogamy, and other abnormalities. Further reduction of the light period by a sufficient decrement below the optimal for sexual reproduction tends to induce intense tuberization. One of the characteristic effects of change in the light period from optimum to sub-optimum for stem elongation is to promote branching. The decreasing length of day of fall is an important factor in causing perennials to enter upon the winter period of dormancy. Abscission and leaf-fall, also, appear to be induced by this shortening of the light period.'

Wanser (8), as the result of his experiments with wheat, supports the views of Garner and Allard, and states that 'the development of winter wheat requires a critical photoperiod for jointing and also a separate and distinct critical photoperiod for heading'.

Schaffner (5) has obtained still more remarkable results from his experiments with Hemp (*Cannabis sativa*, L.). 'In the latitude of Columbus, Ohio, with proper illumination in the greenhouse or in the field or garden, the ordinary varieties of hemp are pure in sexual expression, developing the typical dioecious state when planted at any time between the 10th of May and the 10th of August, and usually also for a week or two outside of these dates. But if the planting is done in the greenhouse between the 20th of August and the 1st of May, sex reversal is almost certain to take place in inverse proportion to the length of daylight at the time of planting and the subsequent period of growth.

'The most remarkable phenomenon observed is the fact that the single factor of relative length of daylight to darkness causes a reversal of the sexual state in both directions. In the staminate plants the reversal is from maleness to femaleness, and at the same time it is from femaleness to maleness in the carpellate plants. The action of the light, therefore, probably goes deeper than merely the reduction of the food supply by reducing photosynthesis.'

Tjebbes and Uphof (7) found that by the use of supplementary electric light at night tulips flowered six days earlier, hyacinths five days earlier, and crocuses nine days earlier than under natural daylight, and that flowering was also accelerated in twigs of Red Currant.

Harvey's experiments (3), already referred to, where the plants were grown under electric light alone, would seem to discount very considerably the conclusions arrived at by Garner and Allard. He states that the 'plants grew well, set good seed or produced tubers in the continuous illumination. It seems unnecessary to have a period of darkness to allow translocation of the assimilate from the leaves. All of the plants in these rooms except cabbage bloomed, and many produced good seed, although the illumination was continuous. It seems then that the period of illumination is not the factor which determines whether a plant will bloom or not. The results obtained by Garner and Allard may have been produced by a modification of the conditions of nutrition of their plants by variation in the length of the day.'

The conclusions arrived at by various experimenters are therefore somewhat discordant. Apparently Garner and Allard attempt to prove too much from their experiments, and it seems quite possible that some of their results are capable of a different interpretation. Their results, however, undoubtedly point to an upper limit of light-duration for certain phases of development in some, but not in all, species of plants. My results with Tomato, Soy Bean, Hemp, and Buckwheat lead to the same conclusion, namely, that increase of light-duration beyond a definite maximum produces little, if any, additional growth. Such plants apparently must have a rest-period. On the other hand the experiments of Harvey with continuous electrical illumination, and the behaviour of plants under continual daylight in the arctic regions, show that in other species of plants healthy growth can be maintained without any period of rest.

Much further experiment will be necessary before any conclusions of a general nature can be drawn as regards the effect of light on (*a*) height, (*b*) weight, (*c*) time of flowering, and (*d*) number of flowers produced. The figures obtainable under each of these four heads must of necessity depend very largely on the amount of photosynthesis carried on in the plant. The amount of photosynthesis again will depend on (*a*) intensity of the light, (*b*) its duration, and (*c*) temperature. It is almost certain that light of lower intensity but more prolonged will produce the same result as light of higher intensity (within certain limits) but of shorter duration. Further, it is highly probable that a higher temperature with light of a given intensity for a definite duration will produce the same photosynthetic result as a lower temperature with light of the same intensity but acting for a more prolonged period. If this reasoning is correct, then the time of the year at which a plant comes into flower must vary within rather wide limits. It is also quite possible that a diminution of the supply of available moisture will have a considerable effect on the time of flowering.

SUMMARY.

Experiments were conducted with 16 different species of plants, including Wheat, Rye, Flax, Hemp, Soy Bean, Tomato, Buckwheat, Sunflower, &c.

Some plants were grown in darkness; others with an exposure to daylight for 3, 5, 6, 10, 12, and 15 hours respectively; while still others were exposed to electrical illumination from nitrogen lamps in addition to full daylight, thus bringing the total exposure to light up to 18 and 20 hours respectively.

It was found that the rate of growth was more rapid at first in the

plants exposed to a diminished supply of light, but at the end those constantly exposed to daylight for a greater number of hours daily ultimately attained the greater height. From the results obtained the deduction is made that the rate of growth, whether in light or darkness, depends on the amount of reserve material available for the formation of new tissue, and that, if two plants have the same supply of reserve material, the one grown in diminished light will make the more rapid growth while this reserve lasts.

The plants grown under a diminished supply of light were deficient in mechanical tissue and had a tendency to become decumbent, or, as in the case of Soy Bean, to assume a twining habit. Plants grown under such conditions, moreover, usually remained unbranched.

The effect of electrical illumination varied in different cases. Where the natural period of daylight from December to March varied from 9 to 12 hours, the addition of an average nightly illumination of 9 hours with lamps varying from 100 to 300 watts had a beneficial result in most cases and promoted the rate of growth and accelerated the time of flowering.

In the plants exposed to daylight for more than 12 hours daily from March to June an average additional electrical illumination of 5 to 6 hours nightly had most effect in the case of Spring Wheat, did not accelerate the time of flowering in Buckwheat or Tomato, and prevented flowering in Soy Bean. As compared with full daylight, electrical illumination in the case of Hemp had a retarding effect on both the height and weight of the plants.

In the case of Tomato, Soy Bean, Buckwheat, and Hemp there appears to be an upper limit to the amount of light which a plant can make use of, beyond which the plant makes no additional growth.

CENTRAL EXPERIMENTAL FARM,
OTTAWA, CANADA.

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The Plant Cuticle.

I. Its Structure, Distribution, and Function.

BY

BEATRICE LEE AND J. H. PRIESTLEY.

(Department of Botany, University of Leeds).

With twelve Figures in the Text.

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1. INTRODUCTION.

IN the present paper the occurrence, structure, and mode of formation of the plant cuticle are examined from a standpoint that should first be briefly stated. All the tissues of a differentiated plant body may be considered to arise from meristematic cells, chemical changes proceeding both in protoplast and wall as these cells vacuolate and increase in size and pass over into the various forms that characterize the adult tissue. When these changes are proceeding in the tissue arising from the apical meristem of the Angiosperm shoot, one of the earliest results is the formation of the cuticle over the surface of the shoot. The meristematic tissues undoubtedly always contain fatty substances (28), which are redistributed during the process of differentiation, much of the fatty acid then appearing upon the walls. In the root the endodermis is then formed, and frequently the exodermis; in the shoot of the Vascular Cryptogam the endodermis is also usually formed (20 and 24), but in the Angiosperm shoot, while an endodermis appears in certain species and especially in shoots growing in special habitats (18), a fat-impregnated endodermis is usually absent, whilst the cuticle is invariably present.

It appears, then, that the formation of a cuticle may be associated with the following three factors:

1. The invariable association of fatty substances, including fats, fatty acids, and lipins (16), with the synthetic activity of the meristematic protoplast.
2. The migration of these substances to plasmatic interfaces during the processes of vacuolation and differentiation.
3. The subsequent migration of these substances into and along the walls separating the protoplasts until they reach the surface of the shoot.

Since Overton (14) first advanced his theory as to the function of lipoids in controlling the penetrability of the plasma membrane, considerable evidence has accumulated of the constant presence of fatty substances in this membrane. Their subsequent accumulation in the wall seems inevitable, and a recent series of papers by Hansteen Cranner (7, 8, and 9) shows conclusively that lipins and fatty acids are present in the plasma membrane, and fatty acids in the wall.

The subsequent movement of these substances in and along the wall is primarily determined by three factors. In the first place, the chemical composition of the wall may provide possible methods of chemical attachment for substances as reactive as fatty acids, and especially unsaturated fatty substances. Secondly, the solubility of the fat compound in an aqueous medium affects its mobility, so that sodium, potassium, and magnesium soaps of fatty acids should move fairly readily along a cellulose wall saturated with water, while calcium soaps would be immobile. On this account the ratio of the bases present in the soil may be expected to affect the thickness of the cuticle. Lastly, many of the fatty substances contain unsaturated fatty acids, which are liable to undergo rapid change in the presence of oxygen, oxidizing and condensing to varnish-like substances insoluble both in water and fat solvents, for which reason intercellular air spaces may affect considerably the accumulation of fatty substances within the tissues.

The invariable appearance of a cuticle in the Angiosperm shoot indicates that, as differentiation begins, these fatty substances move freely along the walls until they reach the surface, where they form a film, which, undergoing condensation and oxidation, gives rise to a rigid layer.

In botanical literature the cuticle is usually said to consist of ‘cutin’. The chemical composition of the cuticle is dealt with in a second paper, but for present purposes it is sufficient to point out that cutin is not a chemical entity; it is a name for an aggregate of substances varying in specific composition, but occurring always at the same place in the plant, and having the same general characters (17). The experimental work described in this second paper supplies evidence in support of the view that cutin is best understood as a mixture of fatty acids with other substances which has undergone oxidation and condensation during its exposure to the air.

From this standpoint the term cuticle can be used with some precision, and at a later stage, when the cuticle of the Filicineae is under discussion, it will be pointed out that it is no longer possible to justify a somewhat more general use of the term in certain classic texts dealing with plant anatomy. By the cuticle is understood throughout a superficial fatty deposit upon the plant, giving definite microchemical fat reactions as described in the next section and distinct from the cellulose layer of the epidermal wall upon which it is deposited. Intermediate lamellae, often called 'cutinized layers', may be distinguished between the cellulose wall within and the continuous fatty deposit without. In these lamellae a cellulose matrix is more or less heavily impregnated with deposits of fatty substances.

In the next section the structure and microchemistry of the cuticle will be considered as a preliminary to a brief discussion of its occurrence in the vascular plant.

2. THE STRUCTURE OF THE EPIDERMAL WALL AND MICROCHEMISTRY OF THE CUTICLE.

As pointed out by de Bary (1, p. 76), in the majority of cases the cuticle may be regarded as a superficial deposit covering the outermost layer of cells, the epidermis, but fairly sharply delimited from it, particularly in young or short-lived epidermal layers where the cuticle does not reach any great thickness and has no cutinized layers (Fig. 1). The epidermal cells form a close-fitting pavement of tabular cells which have each an internal cellulose lamella and a middle lamella, and over which is the fatty deposit, either spread evenly or thickening where it lies over the line of the middle lamella lying between two contiguous epidermal cells.

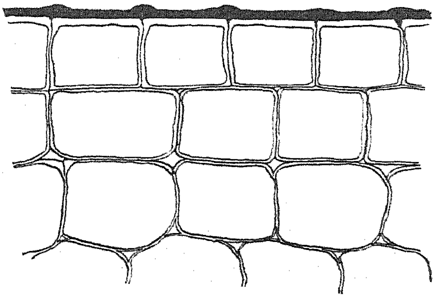


FIG. 1. *Pisum sativum.* Transverse stem of seedling, third node. × 600.

The cuticle shows the sharpest differentiation in microchemical behaviour from the cellulose lamella of the epidermal cell below. Since cellulose is hydrolysed and ultimately dissolved by such reagents as 70 per cent. sulphuric acid, zinc chloride dissolved in hydrochloric acid, and

Schweitzer's reagent, while the cuticle remains practically untouched by their use, the latter can thus be obtained as a thin film on which can be traced the outlines of the underlying epidermal cells.

If hydrolysing agents such as concentrated zinc chloride or 70 per cent. sulphuric acid are only allowed to act for a short time on sections of the epidermis, subsequent treatment with iodine reagents shows the hydrolysed and swollen cellulose layer stained blue, whilst the unswollen cuticle stains yellow. In many cases, this staining reaction is more readily obtained if the sections are first boiled for a short time in alcoholic potash, when a general slight impregnation of fat is removed and the cellulose walls left more accessible to the action of the hydrolysing reagents.

On the other hand, in a young epidermis the cuticle is less resistant to alkaline hydrolysis than the cellulose wall, and can frequently be removed by prolonged boiling in aqueous potash (see Fig. 25 c of *Aloe verrucosa* in de Bary (1), loc. cit., p. 74), still more readily by boiling in alcoholic potash. This is equally true of an old cuticle like that on an old leaf of *Ilex aquifolium*, L., but more boiling is necessary, and the cuticle is made much more resistant if the section is previously treated with sulphuric acid or zinc chloride to hydrolyse the cellulose. The cuticle is, in every case, less resistant to oxidizing agents than the epidermal cell walls, and may be removed from them by soaking in either eau de Javelle (alkaline hypochlorite) or Schultze's macerating fluid (potassium chlorate dissolved in concentrated nitric acid).

Various staining reactions are given for cuticle in text-books of plant histology (see especially Zimmermann (34), Molisch (13), Tunmann (27)). In practice we have found the most satisfactory to be Sudan III, used as a one per cent. alcoholic solution added to an equal volume of pure glycerine. Sections are heated in this reagent on the microscope slide until the alcohol boils, when the fatty layers stain red. With delicate cuticles the layer often melts to form globules before the staining reaction is complete. In these cases use has been made of Nile blue sulphate and of dimethylaminoazobenzene.

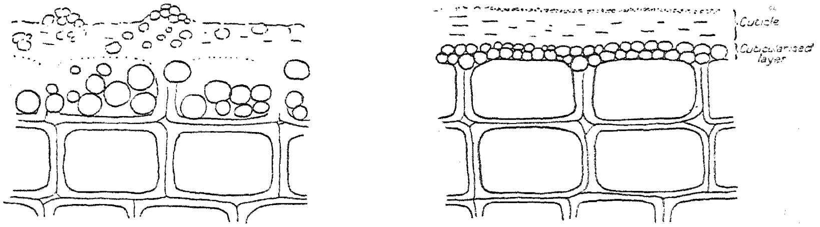
For microtome sections of paraffin-embedded material prolonged staining in an alcoholic solution of cotton red (supplied by Flatters and Garnett, Manchester, under the name of gossypamine) has been found most satisfactory. The cotton red stain is held by the cuticle after it has been washed out of the other tissues by alcohol.

Van Wisselingh (32) has applied to the cuticle wall the same microchemical methods of study as he employed with so much success upon the fat-impregnated walls of cork and endodermis. His experiments, which we have repeated with concordant results, lead to the following conclusions:

1. The cuticular layer is a fat-containing pellicle which is entirely free from cellulose.

2. Unlike the suberin lamella of the cork oak (31), no phellonic acid can be liberated from the cuticle by heating with alkali and then acidifying.
3. Maceration in 10 per cent. potassium hydroxide in either water or glycerine divides the fatty substances in the cuticle into those forming water-soluble globules of soap and those remaining behind insoluble (Figs. 2*a* and 2*b*).
4. By first macerating with potassium hydroxide at ordinary temperatures, and then treating with dilute hydrochloric acid or sulphuric acid, fatty acids melting below 100° C. are liberated from the cuticle. The fatty acids are soluble in alcohol and ether.

Lamarlière has examined the cuticle of various plants (10), using reagents which stain pectic substances. His results indicate the presence of pectic substances most strongly in the epicuticle, i. e. the outermost fatty layer. The pectic reaction diminishes in intensity from this layer towards the cutinized layers and the internal cellulose layer.



(*a*) (*b*)

FIGS. 2*a* and 2*b*. *Ilex aquifolium.* Upper epidermis of leaf. 2*a*. Boiled in NaOH ; the cuticularized layer decomposes first. 2*b*. As in 2*a*, but after further boiling. × 340.

Using his reagents, i. e. ruthenium red, aqueous 1/5000 ; potassium ferrocyanide ; copper ferrocyanide and potassium bichromate, on the cuticles of the leaves and petioles of *Viscum album*, L., *Syringa vulgaris*, L., *Plantago major*, L., and *Crataegus Oxyacantha*, L., this result could not be confirmed in any case. The middle lamella between the cells of epidermis and cortex stained consistently, but in no case did the outermost layer of the cuticle stain strongly, though in some cases the cutinized lamella gave a distinct pectic reaction.

3. THE DISTRIBUTION OF THE CUTICLE.

The synthetic activity of the meristematic protoplast appears to be inseparably associated with the release of a certain amount of fatty substances (16), which are liberated at the surface of the protoplast during the subsequent vacuolation and differentiation of the cell. Probably, therefore, in every relatively massive tissue, fats are available suitable for cuticle formation, but a cuticle is not necessarily formed.

In the Thallophyta, for instance, the majority of the algae are submerged aquatic plants without a typical cuticle. Probably, as the fats reach the surface of the submerged thallus, they form soaps with the inorganic kations present in the surrounding medium. When these are sodium, potassium, or magnesium soaps, they dissolve in the surrounding water, but in predominantly calcareous waters the insoluble calcium soap may accumulate until it forms a characteristic feature of the wall (Pearsall (15), loc. cit., p. 271).

Church ((3) loc. cit., p. 30) makes the statement that 'no such substance as cutin occurs in the equipment of marine phytobenthon, though many plants may show obvious indications of a laminated external membrane, which presents all the general appearance, and may be readily stripped in sheets from the adjacent tissues'.

This is readily understood, as in the submerged algae, although soaps may accumulate at the surface, in the absence of continuous exposure to dry air, the oxidation and condensation of the fatty acids to a continuous, varnish-like membrane cannot be expected.

Many of the large fungi grow normally in air, but very little information is available as to the presence of fatty deposits in their walls. In general a study of the literature suggests that, in the fungi, the fatty substances are usually retained within the cell, the different nature of the wall possibly restricting the extent to which fat migration takes place to the surface of the plant.

With the normal growth of a thallus upon land, as in the Bryophyta, there is less opportunity for the removal of soluble soaps, and more for the condensation and oxidation of the fatty acids. A superficial investigation shows the presence of a marked pellicle, staining with Sudan III upon the surface of thalloid Liverworts and Mosses, and microchemically this layer has all the appearance of a cuticle; macrochemical observations are as yet unavailable.

In the Pteridophyta, the suspension of the stele of *Selaginella* in an air chamber traversed by endodermal trabeculae supplies an interesting demonstration of the dependence of cuticle formation upon air. The cuticular deposit upon the surface of the stele between the trabeculae is continuous and well marked, quite as much so as the cuticle deposited upon the outer surface of the stem.

In the Equisetaceae, the amount of fat present in the epidermis with its siliceous incrustation seems to be slight, and neither carinal nor vallecular cavities are lined with fat deposits. The Equisetaceae, like the Ophioglossaceae, seem to release minimal quantities of fats from the developing tissues.

In the Filicineae, so far as an examination of British species and a few greenhouse plants permits generalization, there are three cases to be distinguished.

In the Ophioglossaceae, a minimal amount of fat seems to be released by the metabolism of the protoplast. The result is that no suberin deposit is ever formed within the endodermis, which contains simply the fat-impregnated Casparian strip (Mager (12)), and the cuticle, though present, is always very thin.

In the Marattiaceae and in the Leptosporangiate Filices, larger supplies of fat are released from the protoplast. In the former, where there is often no endodermis, most of the fats accumulate at the epidermis; in the latter the secondary endodermis, laid down at an early stage of development, is the main repository of fat, while the cuticle remains thin and even in some cases incomplete (Radcliffe and Priestley (24)).

This statement of the distribution of the cuticle within the Pteridophyta differs from that of de Bary ((1) loc. cit., p. 77), who describes *Selaginella* and the Ferns as having the walls of the epidermal cells 'cuticularized all round'. This interpretation is not accepted because the 'brown-walled epidermis' of de Bary does not fall within the category of fat-impregnated layers, but owes its special properties to modifications in the carbohydrate constituents of the wall (see p. 542).

In the Angiosperm the young embryo maturing within the seed is frequently covered by a very thin fat layer, which, with careful staining, is seen to be continuous over cotyledons, plumule, *and radicle.* But immediately upon germination a striking difference is to be noted between the fate of the fats in shoot and root. In the shoot from the beginning they reach the surface, even at the meristematic apex; in the root the fatty substances take longer to reach the surface of the protoplasts and escape to the walls, and in their migration outwards they are invariably stopped before they reach the surface. Some fat is accumulated in the endodermis, but in the majority of roots still more is deposited upon the walls of the exodermis.

This difference in position of fatty deposit can be associated with differences in the chemical composition of the walls of the developing tissues of shoot and root (21 and 28), and is already determined during the maturation of the embryo. The region where transition takes place between the cuticle of the shoot and the surface of the root is usually sharply defined (the collet of Compton (4) and Sterckx), and experiment has shown that when so defined it is impossible to alter its position in some seedlings, as in *Cucurbita Pepo*, L. (Fig. 3).

In *Ricinus communis*, L., there is a long transition region, and by growing the seedling with its stem underground it is possible to increase the numbers of exodermal cells above the hypocotyl. In *Vicia Faba*, L., and *Pisum sativum*, L., there is a similar indefinite area in which cuticle predominates at the upper end, and exodermis towards the lower. The distance at which the cuticle appears down the root can be varied by a few millimetres by growing the whole seedling in the light, and in the case of

Pisum stomata have occasionally developed about half an inch below the junction of the cotyledons where the internal structure is definitely that of the root (Fig. 5). These changes are relatively slight in contrast to those reported by Costantin (6) in his investigation of the difference between underground and aerial stems. In *Vicia Faba*, L., Costantin found that a young shoot grown underground has the walls of its external layers of cells invaded by a brownish red substance which coloured red with fuchsin, as does the cuticle of the aerial stem. Many of his experiments have been repeated, and leave us in no doubt that the changes in the stem, produced by growing it underground, do not include the formation of a typical exodermis which stains red with Sudan III, as in the case of suberin and cutin. In a shoot grown in the soil the cuticle remains thin as in an etiolated stem, until by abrasion with soil particles, and removal of some of the fats as soluble soaps, it is broken down and practically disappears. The superficial cells now undergo considerable change, frequently ending in their death and disintegration, and parallel with these changes their walls discolour, owing to the deposition of acid humus-like substances, which stain with many basic dyes but give no typical fat reactions, and so cannot be regarded as forming exodermal cells.

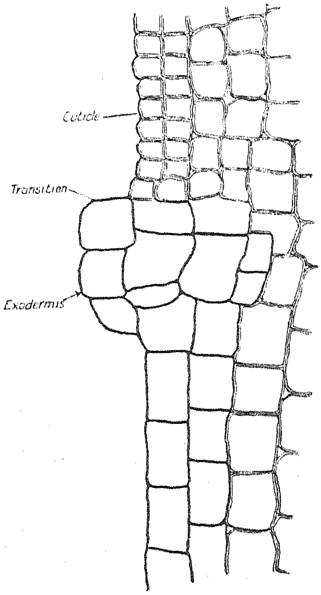


FIG. 3.

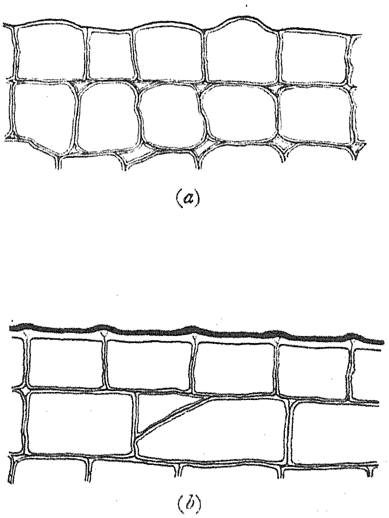


FIG. 4.

FIG. 3. *Cucurbita*. Transition from cuticle to exodermis.

FIGS. 4*a* and 4*b*. *Pisum sativum*. Transverse section of stem. × 400. 4*a*. First node; primary endodermis present. 4*b*. Fourth node; primary endodermis absent.

As previously mentioned, in the Angiosperm stem grown in the light there is no endodermis or exodermis, so that all fats ultimately appear in the cuticle. External conditions can modify this distribution of fatty substances. Although it seems impossible to provide conditions in which a root will grow without forming an endodermis, the shoot grown in the dark, in many species, develops a primary endodermis (Priestley and Ewing (18)), but not an exodermis. Some of the fatty substances being thus arrested in the Casparian strip, it is natural to find that the cuticle is thinner than in the normal shoot. This feature of the etiolated shoot of *Pisum sativum* is illustrated in Figs. 4*a* and 4*b*. It will be seen that the disappearance of the endodermis at the higher level of the etiolated shoot coincides with a thickening of the cuticle.

The cuticle can always be traced right over the meristematic apex of the developing shoot, although thin and easily removed in the youngest region by solution in fatty solvents. As the shoot increases in volume the original cuticle will be greatly stretched, but since it grows in thickness more fat must migrate out to it. Sometimes the addition of additional fat causes the cuticle to wrinkle, the wrinkles appearing first over the radial walls of the underlying cells (Figs. 6*a* to 6*g*). In other cases, presumably because of its continued solution in the substance of the original cuticle, the only sign of the continued addition of fat is the gradual thickening of the cuticle. The following data show the increase in thickness of the cuticle on the stem of *Jasminum* sp.:

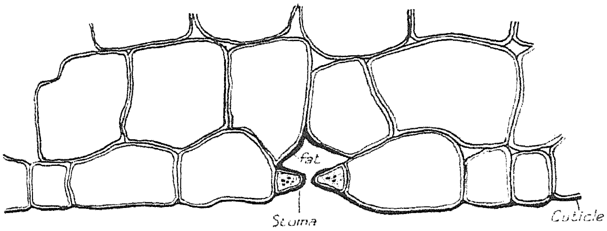


FIG. 5. *Pisum sativum*. Transverse section, half inch below hypocotyl, of root of seedling grown in light. × 600.

A. *Stem of one year's growth, i.e. last year's twig.*

Internodes.	*Thickness in μ of cuticle.*	
1 (oldest)–3	10–12 μ	Fat in subepidermal wall (Fig. 7).
4–11	10	
11–14	8–10	No fat in subepidermal walls.
15	8	

B. *Stem of present year's growth* (July 1923).

Internodes.	*Thickness in μ of cuticle.*	
1 (oldest)	6	Hairs disappear.
2	4–6	
3	4	Hairs present on stem.
4	2–3	
5	1	

As the cuticle grows older it becomes less soluble in fatty solvents, and also loses its power of dissolving additional fat. If after this stage is reached fat still passes out from the interior tissues, it accumulates behind the surface layer in the cuticularized layers, then in the radial walls of the epidermal cells, forming pegs (Fig. 8, petiole of *Ribes rubrum*, L.), and finally, in some cases, as in *Jasminum*, may form a continuous layer of fat completely surrounding the cavity of the epidermal cells, which by this time have probably lost all cell contents (Fig. 7). The above data show how the later inward development of the cuticle takes place after the

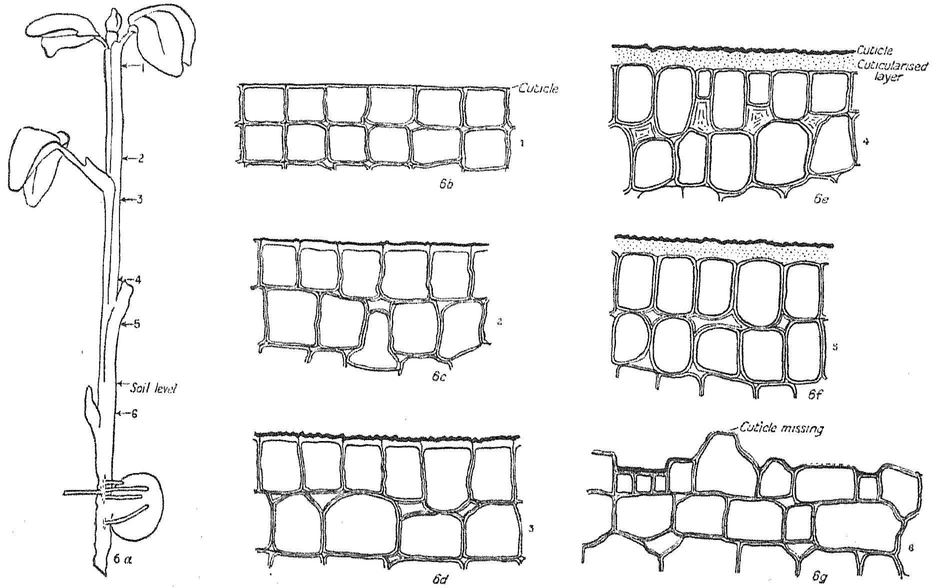


FIG. 6.

FIG. 6*a*. *Vicia Faba*. Seedling. × $\frac{1}{3}$. Primary endodermis present below position marked 6.

FIGS. 6*b–d*. *Vicia Faba*. Stages in the development of the cuticle on the stem of the seedling shown in Fig. 6*a*. The numbers 1–3 correspond with those of Fig. 6*a* and indicate the level at which the section was cut. × 230.

FIGS. 6*e–g*. *Vicia Faba*. Section of stem at lower levels than those of Figs. 6*b–d*. The numbers 4–6 correspond with those of Fig. 6*a* and indicate the level at which the section was cut. × 230.

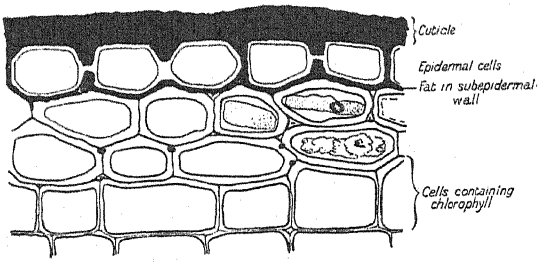


FIG. 7. *Jasminum*. Transverse section of last year's twig at middle of fifteenth node. × 420.

outer layers have practically ceased increasing in thickness, since twelve μ is approximately a maximum thickness for *Jasminum* cuticle.

Another result of the thickening and hardening of the fatty layer illustrated by the *Jasminum* stem is the complete cessation in hair formation. The hairs (Fig. 9) form from an epidermal cell, covered by a thin elastic cuticle, in the youngest internode and remain for a short time. The gap left in the cuticle after the hair collapses is quickly filled in by the rapidly thickening cuticle.

De Bary (loc. cit., p. 29) talks of the epidermis as the layer that *produces* the cuticle. It is true that the epidermal cell frequently loses all its contents, and that the fat it contained will be found in the cuticle ; but the way in which this thickens in many plants indicates that a large proportion of the fat reaches it by migration from inner tissues along the walls. Such migration must take place when walls, and to a certain extent intercellular spaces, are bathed in sap, otherwise the fats would dry and condense upon the walls in passage. Rarely, such a process does take place, with the formation of cuticular patches bordering internal intercellular air spaces (de Bary, loc. cit., p. 215).

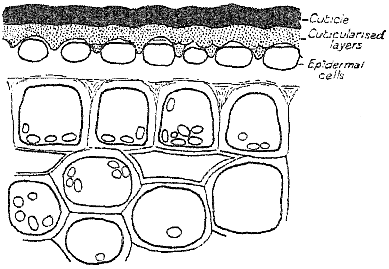


FIG. 8.

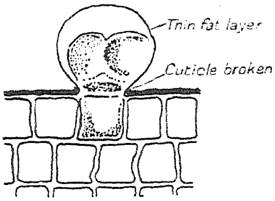


FIG. 9.

FIG. 8. *Ribes rubrum.* Transverse section of petiole ; grown in soil containing no calcium. Note the pegs between the epidermal cells. × 470.

FIG. 9. *Jasminum.* Transverse section of stem, showing capitate hairs. × 500.

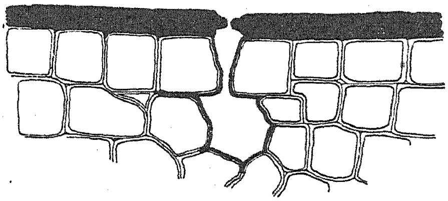


FIG. 10. Peach. Transverse section of stem, showing stoma with fatty deposit lining the walls of the air cavity. × 500.

As the herbaceous stem grows older and bears a larger leaf surface, the loss of moisture from this leaf surface tends to draw off the sap irrigating the cortical tissues from the vascular strands. As a result the fatty deposits accumulate like a tidal deposit as the sap recedes from the walls just below the stomatal cavity (22). Successive tides recur, as exudation pressures increase again at night and diminish in the day, and these gradually lead to the accumulation on the floor of the substomatal cavity of a thin layer of fat resembling cuticle (Fig. 10).

This fat deposit is frequently very thick just at the margin of the guard-cells of the stoma.

Within the superficial region local regions of fat impregnation are very occasionally found, especially in thick-walled cells. A very striking phenomenon is shown by *Vitis vinifera*, L. In the young shoots of this plant no fatty deposits are present in the cortex, but later in the summer there occurs a very wide and diffuse impregnation of the walls of the cortical tissue with fat, and immediately afterwards a pericyclic cork phellogen springs into activity just at the inner limit of the fat-impregnated cortex.

4. THE FORMATION OF THE CUTICLE IN RELATION TO EXTERNAL CONDITIONS.

External conditions will obviously play a large part in determining the nature and extent of cuticular deposits, if these are the result of a chain of processes involving migration of fatty substances on to and along the plant wall, and their subsequent oxidation and condensation at a surface in contact with the air. In the present section, therefore, the influence of external factors will be considered in the light of earlier, and of some additional, experimental data.

External Conditions acting upon Fat Mobility.—The movement of fatty substances to the wall from the protoplast involves the question of the relative permeability of the plasmatic membrane, too large a subject to discuss here, but the data in the literature (Stiles (25)), although by no means always in agreement, suggest that the permeability of the living membrane may be affected by the environment. The subsequent movement of the fatty substances along the wall will partly depend upon two factors: the nature of the fat, and the state of combination in which it is present. 'Fats' can be classified as fats or oils according to their mobility at normal temperatures. Oils, on the whole, are more readily and finely dispersed in an aqueous medium, so that their movement through the framework of a carbohydrate gel is readily facilitated.

The state of combination of the fats in the wall is mainly a question of the relative solubilities of the different soaps formed by the fatty acids. Potassium, magnesium, and sodium soaps are relatively soluble, calcium soaps insoluble. The result that may be expected is to find the mobility of the fat, and, as a consequence, the thickness of the cuticle, controlled by the relative proportion of potassium, sodium, and magnesium ions to calcium ions present in the soil solution. On this point there are not many data available, but, through the kindness of Professor Barker, we have been able to examine the cuticles of the fruit trees used in the manurial experiments of Wallace (29), who has described fully the conditions under which they were grown. Some of the data thus obtained are given below, and certainly support the view that increase in the proportion of potassium is followed by greater mobility of fats and therefore increased thickness of cuticle, whilst increase in calcium diminishes the mobility and results in a thinner cuticle.

Plant.	*Part examined.*	*Thickness of cuticle.*	
Ribes grossularia, L.	Petiole of old leaf.	A. 5·6 μ.	B. 6·0 μ.
Prunus domestica, L.	,, ,,	6·1 μ.	7·0 μ.
Ribes nigrum, L.	,, ,,	9·6 μ.	10·5 μ.

A. No potassium in manure.
B. No calcium in manure.

Note. Each figure is the average of ten different measurements.

The above measurements were made in 1922. In 1923 measurements were made of the thickness of the cuticle of the petioles of leaves taken from bushes of *Ribes glossularia*, L., and *Ribes nigrum*, L., grown under the same conditions as before (see Wallace (29)).

Plant.	*Part examined.*	*Thickness of cuticle.*
Ribes grossularia, L.	Petiole of leaf from seventh node from the tip of the twig.	A. 6·4 μ.
		B. 9·5 μ.
		C. 7·6 μ.
Ribes nigrum, L.	Petiole of leaf from ninth node from the tip of the twig.	A. 4·7 μ.
		B. 5·9 μ.
		C. 4·2 μ.

A. No potassium in manure.
B. No calcium in manure.
C. Receives normal nutrient solution.

Note. Each figure is the average of ten different measurements.

These figures again show that an increase in the ratio of calcium to potassium in the soil results in the formation of thinner cuticle (A), while a decrease results in the formation of a thicker cuticle (B).

As discussed in an earlier section, this ‘basic ratio’ will affect cuticular deposits in another and inverse way where, as in submerged aquatics, these are liable to the continuous leaching out of soluble soaps. This effect was very well shown in some experiments with the cotyledons of *Pisum sativum*, L. The peas were first soaked in water and then slices cut from the cotyledons and the cut surfaces immersed in different solutions as follows:

1. Pure water.
2. 0·1 N solution of calcium nitrate in water.
3. 0·1 N solution of potassium nitrate in water.

After several days' immersion in these solutions the cut surfaces were examined with the following results:

1. Water. Fats were present in the walls of the surface layers of cells. The contents had disappeared from some of the underlying cells.

2. 0·1 N calcium nitrate. Heavy fat deposits were present in the walls of the surface layer of cells. Very little of the cell contents had disappeared.

3. 0·1 N potassium nitrate. No fat deposits were present in the walls of the surface layer. Many of the underlying cells were empty. Evidently the fats had formed potassium soaps and dissolved in the water, and under these conditions, the water having free access to the cells, loss of their contents followed.

Some Potamogetons collected by Dr. W. H. Pearsall from natural waters of known composition were available for examination, and as the results given below show, particularly for *P. pusillus*, the thicker cuticle is in this case found in the water containing most calcium.

Species of Potamogeton.	*Salt predominant in water.*	*Approx. diameter of stem.*	*Thickness of cuticle.*
P. pusillus	Ca	700 μ.	4·0 μ.
P. pusillus	K	500 μ.	1·9 μ.
P. perfoliatus	Ca	2500 μ.	3·7 μ.
P. lanceolatus	K	2800 μ.	1·2 μ.

The presence of a cuticle on an aquatic Angiosperm may seem at variance with the explanation given above for its absence from submerged Thallophyta, but the former is a plant with a continuous system of internal air spaces, in which the air is practically in equilibrium with the gases dissolved in the surrounding water, and therefore with the external air. Under these conditions the fats accumulating at the surface are probably fairly readily oxidized, though the cuticle must always remain relatively little condensed and subject to loss by solution of soaps into the surrounding medium.

External Factors and Cuticular Changes.—When the fats have reached the surface of the plant, light exerts an effect on the chemical changes proceeding in the fat, though the mechanism of its action is as yet unknown. The changes are, presumably, photocatalytic and relatively rapid in nature.

One of the fatty acids derived from suberin, phellonic acid, gives a very characteristic reaction with iodine and sulphuric acid, and Miss Rea observed, in this laboratory, that after a few hours' exposure to the daylight this reaction disappeared. In the case of cutin, no fatty acid with such a characteristic reaction has been isolated to permit experimental investigation of the action of light, but the following experiment certainly suggests that the oxidation, and probably the condensation, of the fatty acids is facilitated by light.

Cellulose plates were made by dissolving filter-paper in Schweitzer's reagent and reprecipitating the cellulose with hydrochloric acid. After thoroughly washing the precipitate free of copper and acid, and compressing it into plates, a film of oil extracted by chloroform from radicles of *Vicia Faba*, L., was then spread over three such plates and they were left:

1. In sunlight in a closed Petri dish.
2. In the dark in a wooden box in a closed Petri dish.

3. Under a bell-jar in darkness, in an atmosphere of coal gas.

After leaving for several days sections of the cellulose disc stained with Sudan III showed in:

1. A firm film of oil not melting when the reagent was heated on the slide until the alcohol solvent boiled, firmly attached to the cellulose and very resistant to the action of such fat solvents as ether and chloroform.
2. The fat layer was more readily dissolved and less attached to the cellulose.
3. The layer of oil was very little changed from its condition when placed on the cellulose.

The displacement of oxygen by coal gas in 3 has effectually prevented the oxidation of the oily film, but a comparison of this with the other two suggests strongly that light has accelerated the rate of oxidation as with the fats in the cuticle, and that subsequently the layer of oxidized fats has condensed to a varnish-like film, firmly adhering to the cellulose.

Wm. S. Cooper (5), working on the broad sclerophyll vegetation of California, has measured the thickness of the cuticle on the upper and lower surfaces of leaves taken from habitats of increasing moisture and decreasing light intensity. The results of averaging his measurements of many cuticles are given in the following table:

		Upper Cuticle.	*Lower Cuticle.*
Light intensity decreasing with increase in moisture from 1 to 4.	1. Climax chaparral	6·36 μ.	5·23 μ.
	2. Broad sclerophyll forest	4·31 μ.	2·06 μ.
	3. Redwood forest undergrowth	3·52 μ.	2·04 μ.
	4. Deciduous species	1·58 μ.	0·91 μ.

From the results of his work he concludes that the thickness of the cuticle varies as the decrease in moisture and the increase in light intensity.

It is true that for the same plant grown under these different conditions, the cuticle would thicken as the air dried and the light increased, but this generalization cannot be applied to all plants, since other factors are concerned.

Plants vary in the amount of fat they contain. Heath plants synthesize large quantities, and this results in the formation of very thick cuticles on their leaves and stems from the young stage onwards. On the other hand, a plant that has little fat in its metabolism can only form a thin cuticle.

That oxygen is necessary for the proper formation of the cuticle is undoubtedly true, but confirmation by experiment with the growing plant is difficult to obtain. The original fat deposit at the surface of a tissue is in every way comparable with the fat deposit, usually termed a suberin layer, which precedes cork formation at the surface of injured tissue, and in this case the experimental evidence is conclusive in favour of the need of oxygen for its formation (Priestley and Woffenden (22, 23)).

That oxidation is proceeding during the formation and maturation of a cuticular deposit is suggested by the low iodine numbers obtained for the chloroform soluble fats from the cuticle (see second paper) compared with such numbers as Lewkowitsch gives for vegetable oils (11).

Other evidence for this oxidation process is given by a comparison of the iodine number of the chloroform soluble fat of the cuticle of outdoor-grown rhubarb and that of the cuticle of forced rhubarb grown in warm, dark, damp sheds under conditions unfavourable to condensation.

	Iodine number.
Outdoor grown	40–43
Forced rhubarb	90–91

The oxidation processes involved in the cuticle will be complicated in nature, but undoubtedly light is thrown upon them by investigations such as those of Coffey (2). Oxygen probably enters at the double bond, and further oxygen taken up leads to the splitting off of volatile acids and water. Traces of acids of low molecular weight have been found in the cuticle, as will be described in the second paper. The loss of water resulting in the further condensation of oxyacids to the peculiarly resistant 'linoxyn' class of substance is the reaction which seems mainly responsible for the production of the most characteristic properties of cuticle, and explains why the hardening is so very dependent upon the humidity of the air.

As to this fact there is no doubt. Tittmann (26) noticed the phenomenon in his experiments upon regeneration of cuticle, describing the regenerated cuticle as 'weaker' when formed in a moist atmosphere under a bell-jar. The phenomenon is well shown in etiolated plants where differences in light are excluded. With many species, if these are grown in a moist atmosphere, the endogenous root initials freely formed at the nodes are able to burst through the soft cuticle, but with the same cuticle formed in a dry atmosphere these show only as white patches, slightly pushing up the relatively tough cuticle, but unable to penetrate it. This lack of resistance on the part of the 'soft' though not necessarily thin cuticle formed in a very moist atmosphere, to internal swelling forces due to turgid tissues, is one of the factors that has to be considered in arriving at a causal explanation of all proliferations involving epidermal tissues, as in the many forms of intumescence reported at different times.

5. THE EFFECT OF THE CUTICLE UPON THE PLANT.

The fact that the cuticle covers the Angiosperm shoot from its earliest stages means that it will have certain effects upon its course of development.

Water is probably slightly soluble in the original fatty acids, but penetrates with less ease the linoxyn-like products that result from their drying. As might be expected, therefore, the amount of water vapour lost from the plant through the young cuticle diminishes as the cuticle gets older. These facts require little experimental support, but the following data, obtained with forced and outdoor-grown rhubarb petioles, are cited (particulars of the nature of the cuticle on such petioles will be found in the second paper).

Ten pieces of outdoor-grown and ten pieces of forced rhubarb, with a diameter of 1·2 cm. and a length of 3·5 cm., were cut and their ends sealed with Spenwood's liquid cement to prevent loss of water. These were put into desiccators and left to dry over anhydrous calcium chloride. They were weighed every day. The numbers are an average for the weights of ten pieces, and give the differences of weight between each day's weight and the original as percentages of the original weight (Fig. 11).

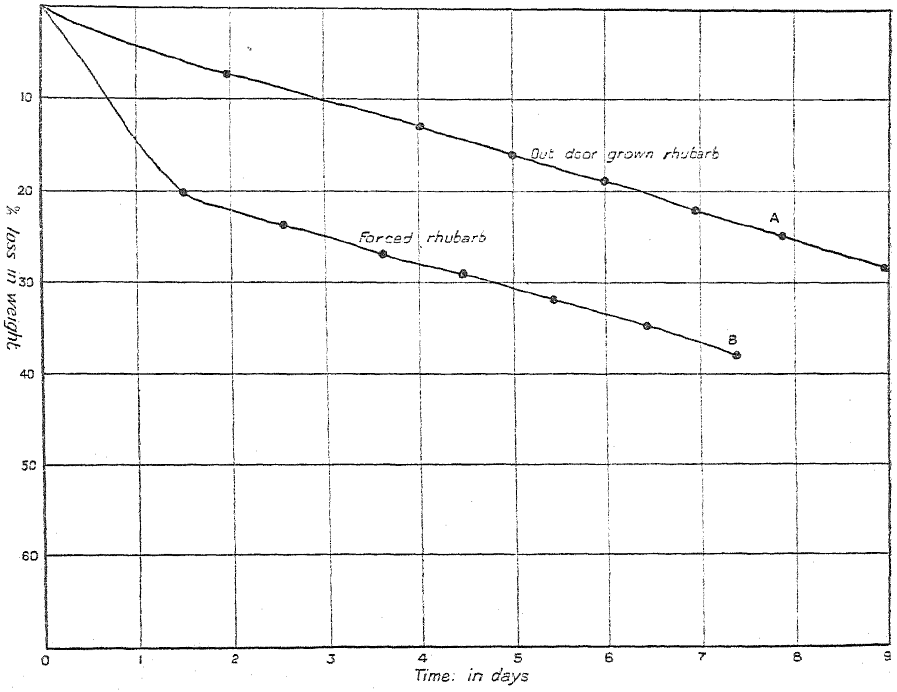


FIG. 11. Graph showing relative rates of drying of A, outdoor-grown rhubarb; B, forced rhubarb.

	% *Loss in weight.*		
Time of weighing.	*Outdoor.*	*Forced.*	
Outdoor	7·32	20·11	
Every day at 6.0 p.m.	12·02	23·87	*Average weights*
For eight successive days	15·26	26·15	*of pieces.*
	18·21	29·33	Outdoor = 4·1 grm.
Forced	21·49	32·03	Forced = 3·6 „
Every day at 10 a.m.	25·51	35·11	
For seven successive days	28·83	38·44	
	31·63		

These data show that loss of water from the cellulose wall underlying the cuticle must be considerably impeded by its presence. In the next paper it will be shown that these cellulose layers just beneath the cuticle, probably owing to their long exposure to light in a relatively dry atmosphere, have so far undergone condensation that about 30 per cent. of the cellulose resists hydrolysis by prolonged boiling in 10 per cent. sulphuric acid. If the cuticle had been absent it is probable that the desiccation of the cellulose would have gone still farther, and it is suggested that it is exactly this condition of affairs that explains the development of what de Bary calls the brown cuticle of the Leptosporangiate Ferns. Here the original fat deposit is so slight that it can hardly be traced in the adult rhizome, but chemical condensation has proceeded so far in the walls of the outer layers that they are composed of an extremely resistant cellulose that, in addition, is impregnated by condensation products of tannins, giving it its dark brown colour.

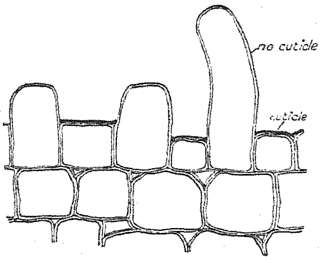


FIG. 12. *Pisum sativum.* Transverse section of stem of seedling taken at a point two inches below the surface of the soil. Note the appearance as of root-hairs of some of the epidermal cells whose cuticle is broken.

At quite an early stage in the growth of the Angiosperm the layer of cuticle is changing from an oily film to a resistant varnish. Consideration suggests that the time of this change will be of great importance in reference to the development of epidermal structures. The varnish-like stage is resistant to stretching. Willows and Alexander (30) show that when portions of a mature cotton hair are soaked in alkali, the cellulose swells out at the cut ends of the length of hair, instead of forcing up the cuticle. They conclude that 'the force required to move a substance like cotton cellulose along a narrow-bore tube of the diameter of the fibre must be enormous', and that this 'demonstrates the great rigidity and strength of the cuticle'.

The cuticle on the leaves and stems of most plants grown under normal conditions is sufficiently rigid to maintain a regular tabular form in the cells of the epidermal layer by preventing their extension, while the epidermal cells of an etiolated stem, or a stem grown underground, are large and irregular, in some cases resembling root-hairs (Fig. 12). The epidermal cells of a seedling of *Pisum sativum*, L., grown with four inches of its stem underground so that it was under root conditions, grew out to form 'root-hairs', since no resistance was offered to them by the cuticle.

Obviously these considerations might throw light upon the occurrence of hairs upon the epidermis, the formation of the hair requiring the extension of the epidermal cell at a stage when the cuticle is still uncondensed. Such considerations can be applied to differences of hair pattern as described by Yapp (33) in *Spiraea Ulmaria*, where the hairs, instead of being spread over the whole of the lower leaf surface, in many leaves are restricted to a region in between the veins. Teleologically, this can be explained as being a region where excessive transpiration is most effective and first produces indication of withering. On the other hand, during leaf development, such regions midway between the veins are probably less exposed, so that the cuticle dries at a later period in development. This slowness in development of cuticular rigidity might be responsible for the hair pattern developed by the leaf.

It is particularly in the leaf with its large surface in proportion to its mass that the cuticle may prove an important factor in causal anatomy. In other work in this department, we have been led to consider an early and thick cuticle as associated with a small and but slightly expanded leaf (Priestley and Hinchliff (19) on peat plants), and in conclusion we would tentatively raise the question as to the possible relation of the typical histology of the leaf to this factor. Strongly insolated leaves always have palisade parenchyma stretched at right angles to the surface. This is usually regarded as being directly connected with the light incidence, but it is open to question whether the fact that, in such a leaf, further lateral stretching of the cuticle ceases to be possible at an early stage in development may not have the result that, when the palisade cells stretch, their elongation perforce takes place at right angles to the resistant cuticular surfaces.

6. SUMMARY.

1. The development, structure, and distribution of the cuticle is studied from the standpoint that the fats migrating from the protoplasts of both external and internal tissues may find their way on to the walls during differentiation and then along the walls to the surface, where they contribute to the cuticle.

2. Microchemically the cuticle is characterized by its capacity to stain with fat stains, its great resistance to acid hydrolysing agents, and its ready oxidation by alkaline or acid oxidizing agents.

3. The cuticle forms a continuous layer outside the cellulose wall of the epidermal cell; beneath it may be present cellulose 'cutinized lamellae' impregnated with fatty deposits.

4. The cuticle is normally present on the surface of leaf and shoot of the Bryophyta and all vascular plants. Certain peculiarities of its distribution in the Pteridophyta are briefly indicated.

5. The continuous development of the cuticle in the Angiosperm shoot is described, and supplies evidence that the cuticle is thickened by fatty substances migrating along the walls from interior tissues. Its absence

from the root is determined by differences in wall structure and development, already present in the growing points in the embryo and little affected by external conditions during germination.

6. The mobility of the fatty substances in the walls may be modified by external conditions which thus affect the thickness of the developing cuticle. This thesis is supported by reference to the cuticles on leafy shoots supplied with varying ratios of potassium to calcium.

7. On the other hand, in submerged water plants the presence of a high proportion of potassium to calcium produces a thin cuticle, because the soluble potassium soaps leach out into the surrounding water.

8. Light and humidity affect the thickness and consistency of the cuticle by their influence upon the oxidation and condensation of fatty acids, processes involved in cuticle formation.

9. The presence of the cuticle influences the rate of evaporation from the plant, and thus, indirectly, the degree of condensation undergone by the cellulose wall beneath. This consideration appears to throw light upon the 'brown epidermis' of the Ferns.

10. The cuticle resulting from chemical condensation in dry air is very resistant to stretching forces. As a result the rate of condensation during cuticle development may affect the appearance of epidermal outgrowths such as hairs.

11. It is suggested that the subsequent extension of parenchymatous tissues, such as the palisade parenchyma of the leaf mesophyll, may be affected in direction by the resistance to stretching of the cuticular surface.

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On the Structure of Spyridia filamentosa, (Wulf.) Harv., and the Affinities of the Genus.

BY

REGINALD W. PHILLIPS, MA., D.Sc.,

Emeritus Professor of Botany in the University College of North Wales, Bangor.

With ten Figures in the Text.

'AMONG the better-known forms of Florideae, there is scarcely a genus concerning the true affinity of which do I find to-day more diverse opinions expressed than *Spyridia*.'

So wrote J. G. Agardh in 1897, in his eighty-fourth year, when he returned to a consideration of the genus after more than one previous discussion (Agardh, J. G., 1851, 1876, 1880, 1897).

The position assigned to *Spyridia* by modern writers is that in which Schmitz placed it in 1889 in his 'Systematische Uebersicht der bisher bekannten Gattungen der Florideen', where the Spyridieae form one of the fifteen subdivisions of the family Ceramiaceae.

The criterion upon which Schmitz based his system of classification was, first and last, the development of the cystocarp from its inception to its maturation. Schmitz had not himself investigated the case of *Spyridia* at the time of the publication of his 'Untersuchungen über die Befruchtung der Florideae' in 1883, but in the diagnosis of the group Spyridieae by Schmitz and Hauptfleisch in Engler and Prantl's Pflanzenfamilien (1897) there does appear some account of the procarps and ripe cystocarps, though it is only slight and inadequate, and without illustration of any kind.

Under these circumstances it seemed to me that it might be useful to make a careful examination of the type-species *S. filamentosa*, which grows freely on the coast of Anglesey, and to give such an account of the structure of the thallus, and particularly of the cystocarp, as might fill up some of the gaps in our knowledge of the genus, and perhaps throw more light on its systematic position.

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The geographical distribution of *S. filamentosa* and of the other 17 species which have been admitted by Agardh (1897) into the genus seems to indicate that it belongs to an ancient stock, well marked and widely spread.

The coast of Anglesey is the most northern station in which *S. filamentosa* has been found. It appears afterwards on the south coast of England and the coast of France, then in the Mediterranean, the Red Sea, and the Indian Ocean. It is plentiful on the Atlantic coast of North America, from New England to the West Indies. It also occurs at the Cape.

Among localities from which other species of *Spyridia* are reported are Senegambia, Australia, New Zealand, and the Sandwich Islands. Schmitz and Hauptfleisch express the opinion that some of the species described by Agardh may be found to belong to other genera, but as the vegetative structure and the form of the cystocarp in the type-species are very characteristic, and as all the other forms were carefully compared with it by so great an authority as Agardh, with the experience of years, it is hardly likely that they do not fall into the same genus with the type. It must also be remembered that Agardh had already discarded as many as 17 out of the 22 species of Kützing (1849).

S. filamentosa, like most other Florideae, occurs in three forms, viz. male, female, and tetrasporiferous. It is, to use the term introduced by Svedelius (1915), diplobiontic, and the sexual and asexual generations will no doubt be found to alternate regularly. It exhibits what Oltmanns (1904) has called the 'central-thread' type of structure in contradistinction to his 'fountain' type. Outwardly, it somewhat resembles *Polysiphonia*, in that each cell of the central thread appears to be surrounded by a number of elongated cells looking very much like the 'siphons' of that genus; inwardly, it is more like *Ceramium*, in that each cell of the central thread is distended into a more or less spherical contour. It is, however, different in structure from both these genera.

In the tufts of *S. filamentosa* it is easy to distinguish between axes of limited growth and those of unlimited growth.

The former are the 'filaments' to which the species owes its specific name, given by Wulfen and adopted by Harvey. Arising, apparently, one from each cell of the axis, close behind the apex, these filaments at first possess a typical apical cell of their own, which ceases, however, to function as such when it has cut off about a score of axial cells. It then becomes transformed into the acuminate apex of the appendage, reminiscent of a similar condition in the whorled appendages of *Chara*. The three or four cells near the base of the filament are small and without cortex. Those farther up are broader, and are imperfectly corticated by the production of a circlet of small cells from the upper rim of the axial cells. This condition very closely resembles that of all the axes of the incompletely corticated species of *Ceramium*, and predisposes the observer to see at first a relationship between *Spyridia* and that genus, which is not confirmed by further observation. *Ceramium* has really no such appendages as *Spyridia*, which are limited in growth and later fall off. Some species of *Polysiphonia*, on the other hand, possess lateral appendages, which have been called 'leaves', but which possess no cortication and drop off very early.

The function of these appendages is no doubt assimilatory, and they thus constitute the nearest approach to leaves in both structure and function. Their insertion in the main axes by a narrow basis makes them sway with every movement of the water. It also facilitates their fall when they have served their turn. Like leaves, they are crowded on the younger parts behind the apices, while the older parts are completely divested of them.

In their limited growth and attenuated apex these filaments are somewhat like the lateral appendages of *Dasya*, but there again they are without cortex and are branched (Phillips, 1896).

Among the axes of unlimited growth it is possible to distinguish between main and subsidiary. *S. filamentosa* is caespitose in habit, tufts of freely-branched axes arising from a common spongy cushion of rhizoids attached to the solid substratum. The main axes grow with greater vigour, the lateral subsidiary axes with less active growth in length, not a difference in kind, but just the difference between the leading and lateral branches, say, of an elm tree. Agardh has tried to find in *Spyridia* axes of three kinds—rami, ramuli, and ramelli. The ramelli are the filaments, but I can see no essential difference between rami or main axes, and ramuli or subsidiary axes.

I do, however, find a difference among ramuli or subsidiary axes. First there are those which arise in regular acropetal succession behind the apex, and those which arise later mostly in parts denuded of filaments, in no regular succession, and which might be called adventitious. It is such branches that give rise to procarps, and later become transformed into the cystocarps.

It will be remembered that in *Rhodomela subfusca* and *Polysiphonia elongata*, after a period of vigorous vegetative development, the plant is stripped of its smaller vegetative axes, and later buds forth numerous adventitious branches; upon these, the reproductive structures, male, female, and neutral, are crowded. In *S. filamentosa*, while the antheridia and tetrasporangia are found on the axes of limited growth solely, the procarps are found, at any rate mostly, on these adventitious branches of unlimited growth. That is to say, the condition which is more or less fixed in the two Rhodomelaceae is found in an incipient stage in *Spyridia*. Fertilization failing, I believe that these fertile adventitious branches may grow forward in a purely vegetative fashion.

At the apex of one of the vegetative axes of unlimited growth, there will be observed a conspicuous dome-shaped apical cell, from the base of which cells are cut off in the form of very thin circular discs which are concave forward. From the last-formed disc back to the older parts, every step in the curious mode of cortication occurring in the genus may be made out. The first segment to be cut off from one of the axial cells immediately behind the apex is one which gives rise to the appendage of limited growth. Then, according to Cramer (1865), as many as 13 other cells are cut off peripherally in a remarkable order of succession, right and left of the segment bearing the appendage, until the whole periphery is covered with pericentral cells, or what Cramer calls primary cortical cells. The figure displaying this curious scheme of segmentation is reproduced by Oltmanns (1904). Cramer also claims to have made out that the angular distance between first segments cut off from successive axial cells, i.e. between successive appendages, is $\frac{5}{13}$ of the periphery, so that the 14th appendage stands vertically above an appendage numbered 1. These 14 cells ultimately form a circlet round the upper end of the axial cell when it has become elongated by growth, as the elongation seems to take place mainly in the basal part of the cell. As this elongation proceeds, each of the 14 primary cortical cells of Cramer buds off two cells below, which lengthen *pari passu* with the axial cell and set up a secondary pit connexion with the alternating cells of the circlet next below, so that each primary cortical cell above is joined by these means with two such cells of the series next below. These cells, about 28 in number, joining the cells of one circlet to those of the next, eventually attain a length many times as great as that of the primary cells, which hardly lengthen at all, and constitute the system of 'siphons' which makes *Spyridia* superficially somewhat resemble a *Polysiphonia*, although the 'siphons' of the latter genus arise in a quite different manner. In *Polysiphonia* the 'siphons' abut against those of the next articulation, in *Spyridia* they are interrupted by the circlet of short cells at the node consisting of just half the number of the 'siphons'. In no *Ceramium* either, does the cortication at all resemble the first stage of cortication in *Spyridia*, for in that genus the primary cortical cells bud above, as well as below, and in no such regular pattern as in *Spyridia*. A single joint of *Spyridia* consisting of a spherical central cell crowned with 14 primary cortical cells, and invested with 28 secondary siphons, no doubt suggested the name, from Gr. *Spyridion*—a basket.

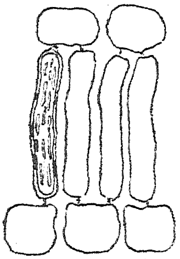

FIG. 1. Showing pattern of first cortication on main axes of *S. filamentosa*. × 100 *ca.*

This first stage of cortication in *Spyridia* becomes much obscured in the older parts by a copious growth of hypha-like cells, which arise from both the primary and secondary cortical cells. These cells insinuate themselves in the common mucilaginous wall between the cells of the original cortex, and later on, even outside it, so as quite to obliterate the original pattern and to add greatly to the thickness and therefore strength of the axis. When, however, such an axis is cut across in the older parts, the original 28 siphons may be counted approximately round the axial cell, with numerous sections of superposed rhizoid-like cells on the outside. If, however, the section is made at the node, the half-number of primary cortical cells may be counted, similarly superimposed with hypha-like threads.

To those who look in the structure of the thallus for indications of affinity, there is no safe clue forthcoming from a study of *Spyridia*. Its method of cortication of the axes of unlimited growth is peculiarly its own, and though the cortication of the axes of limited growth certainly resembles that of *Ceramium*, it occurs upon structures which have no exact counterpart in that genus.

Before leaving this reference to the vegetative characters, I might mention the curious trichomes which belong to *S. filamentosa*. They are found mostly on the cortical cells of branches of limited growth, and bear a curious resemblance to the trichogynes, but seem to arise on any part of the exposed surface of a cell and often more than one on each. They cannot be detected at all in dried material, and it is only in well-fixed material carefully handled that they can be properly made out. They are extraordinarily attenuated and of great comparative length, but in stained material the thin thread of protoplasm, continuous with that of the cell upon which they arise, may be traced throughout. In the form named by Kützing *S. villosiuscula*, it is no doubt these trichomes which give the villous appearance to the cortex of the filaments. Agardh does not admit *S. villosiuscula* to specific rank, but merges it into *S. filamentosa*, and certainly the type-form is not devoid of them. I have thought that their function is respiratory. If they are growth-forms, it would be interesting to ascertain the conditions under which they grow in such profusion as in Kützing's plant.

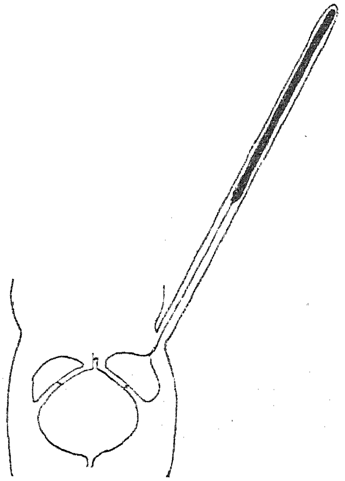

FIG. 2. Showing young trichome on a cortical cell of a filament of *S. filamentosa*. They are several times as long when mature. × 250 *ca.*

The antheridia of *S. filamentosa* have been described and figured by Farlow (1879) and Buffham (1888). I do not find that the condition affords any clue to relationship. Like the tetrasporangia, they occur only on the filaments or axes of limited growth. The fact that the tetrasporangial mother-cell protrudes from the ring of cortical cells, even before the tetraspores appear, constitutes a contrast to the condition in *Polysiphonia* and *Ceramium*, where the tetrasporangia remain immersed until the maturation of the spores.

To come now to the development of the cystocarp, which is, by general consent, the critical matter for purposes of classification, I would point out that I have already identified the axis which becomes transformed into the cystocarp as an adventitious axis of unlimited growth. It is, to use the terminology of Agardh, a transformed 'ramulus' and not a transformed 'ramellus'. Like the filaments, they arise at the nodes, but are seated more firmly on the supporting axis, with a strengthening cortication at their very inception.

If now the apex of such a branch, which has produced about a score of axial cells, but which is nevertheless still very minute, be examined with a high power it will be found already to have produced one or more, sometimes a series of procarps (Figs. 3, 4, and 8). From the apical cell there are cut off cells which are destined to become alternately what we may call sterile and fertile axial cells. By a sterile axial cell is meant one which does not give rise to a procarp, and a fertile axial cell is one which does. If an axial cell is destined to be sterile, it cuts off first a cell which gives rise to an axis of limited growth, i. e. a filament, and afterwards a whorl of cells, which to the number of 7 or more grow outwards to form in course of time a branching system. These cells no doubt correspond to the primary cortical cells of the vegetative axis, and the branched systems derived from them to the immersed cortex. Here however the branches radiate outwards in the common distended mucilage, though still immersed. If an axial cell is destined to be fertile, it cuts off 4 pericentral cells (corresponding again to the primary cortical cells), one of which gives rise to a 4-celled carpogonial branch. This branch with the supporting pericentral is what is termed the procarp. I have figured a case of such a procarp-bearing axis (Fig. 3), the 2nd cell of which (counting the apical cell as number 1) is undivided; the 3rd cell has given rise to the first 2 cells of a branch of limited growth; the 4th cell has cut off a pericentral cell which is initiating a carpogonial branch; the 5th cell is sterile and has produced an appendage of 6 cells overtopping the apex; the 6th cell is fertile, with a carpogonial branch, the trichogyne of which has emerged beyond the surface; the 7th cell is sterile, has produced an appendage of 15 cells as well as several pericentral cells; the 8th cell is fertile, with 3 undivided pericentral cells and 1 with a carpogonial branch and a greatly elongated trichogyne; the 9th and 10th axial cells are respectively sterile and fertile. I have found an even greater number of procarps than 4 as figured, and believe that when fertilization is delayed the number may be considerable. It will be observed that the axial cells which give rise to procarps are sensibly smaller than those which give rise to sterile branches, and it is possible to detect this alternation of size at a point below that at which the oldest carpogonial branch can be distinguished among the luxuriant sterile growths. I have tried to determine the order which is preserved in the succession of carpogonial branches, and in the succession of branches of limited growth, but though it is certain such an order exists, I have not been able to make it out. The trichogynes emerge on all sides, and the carpogonial branch has the same curious double curvature which is found elsewhere. With regard to this curvature, while it doubtless serves to keep the carpogonium within easy reach of the point of origin of the branch, it probably is brought about by the circumstance that while the base of the branch is a fixed point, so also is the point of emergence of the trichogyne, and the enlargement of the cells of the branch between these points involves a curvature which is the greater the more they enlarge. Looking down on the cells of this branch from the surface, they seem to overlie one another in a crowded curve.

With regard to the fertilization, it may be asked whether more than one carpogonium may not become fertilized on the same axis. It is probable that this is so, but I have never been able to discover, in a more mature cystocarp, evidence that more than one act of fertilization is effective in the production of carpospores. This is probably due to the fact that a great demand for nutritive material is involved in the consequences of fertilization, so that even if a second fertilization occurs nothing comes of it after the initial stage. The same question arose in the case of *Delesseria sanguinea*, where the procarps were much more numerous, but there also I found that only one fertilized procarp matures in the cystocarp.

The first effect of fertilization is no doubt here as elswhere the fusion of the male and female nuclei in the carpogonium, though the methods of treatment which I have adopted have not admitted of direct observation of the nuclear movements (Fig. 4). A secondary effect can, however, be observed in all cases. It is the formation of an exceptionally large auxiliary cell with which the carpogonium soon conjugates. This cell is either the pericentral cell supporting the carpogonial branch or a cell derived from it on the upper side towards the carpogonium. Analogy with many other cases would seem to suggest that it is the latter alternative which holds good, but, if so, the auxiliary cell itself is so voluminous that the pericentral from which it is cut off must be small by comparison; and although I have, as I think, made out the existence of this smaller residual cell in some of my preparations, it is not easy to distinguish it in all cases, and I am inclined to think it may fuse again with the auxiliary at a later stage. The auxiliary cell lies over the shoulder of the axial cell within the curve of the carpogonial branch, in close contact with the carpogonium, with which I have found it in open communication. Its appearance at this stage is quite remarkable after treatment with glycerine. The wall is unusually thick, as are also those of the cells of the carpogonial branch, so that the group of cells, consisting of the pericentral cell, the auxiliary, and the cells of the carpogonial branch, seems isolated among the surrounding tissue by a common swollen mucilaginous investment.

While the fertilization of the carpogonium and its conjugation with the auxiliary are taking place, the three other pericentral cells of the fertile joint begin to develop, though this is long after the pericentral cells of the sterile joints above and below have all developed a copious branching, overarching a space opposite the fertile joint, unoccupied as yet save on the side on which the procarp lies (Fig. 4). Within this space the three pericentral cells now divide into a large outer cell and a small inner one. The large outer cells now grow a beak-like conjugation process in the direction of the auxiliary cell, with which they are by this means put into open communication. This is easy in the case of the two cells which lie right and left of the auxiliary, but seems difficult in the case of the third, which lies on the opposite of the axial cell away from the auxiliary. I have satisfied myself, however, that this conjugation with the auxiliary takes place in the case of

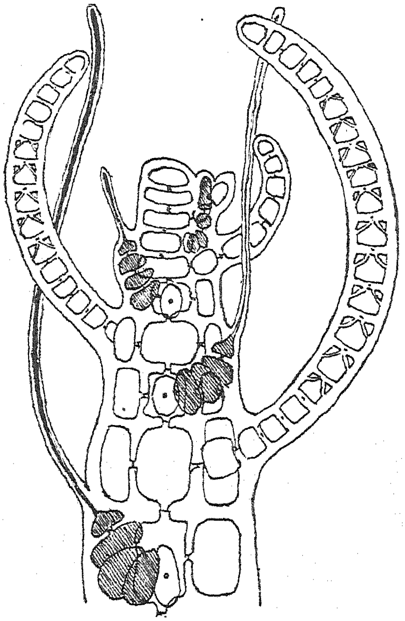

Fig. 3.

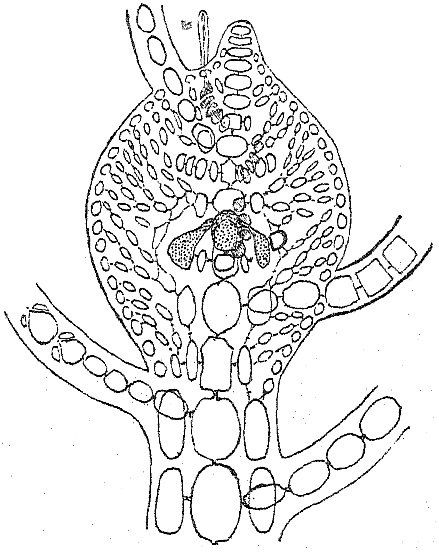

Fig. 4.

FIG. 3. For description see text; semi-diagrammatic, adapted from drawings taken by means of the camera lucida. The walls of the cells are greatly swollen by treatment with glycerine, and the protoplasts in consequence stand much farther apart than they do in nature. The carpogonial branches are obliquely hatched. × 400 *ca.*

FIG. 4. Semi-diagrammatic. The procarp on the eleventh joint from the apex has been fertilized, the primary auxiliary (dotted) cut off from the pericentral cell, and the three secondary auxiliaries (of which only two—dotted also—are shown in the figure) have put out conjugation tubes (haustoria) reaching the primary auxiliary. On the right the secondary auxiliary has budded off the first cell of a gonimoblast fascicle. × 200 *ca.*

the second cells derived from all three pericentrals. The conjugation tube derived from the cell opposite the auxiliary reaches clean over the shoulder of the axial cell. It would seem as if the auxiliary cell exercises a chemitropic influence, calling forth the formation of these tubes, and the appearance presented at this stage is very remarkable and unlike anything which has yet been described in other Florideae.

I may anticipate here by stating that it is these three cells which put out conjugation tubes, and these alone which later give rise to the gonimoblast filaments upon which the carpospores arise.

It would seem, therefore, that they must be regarded as auxiliary cells. If so, there are four auxiliary cells, one primary, conjugating with the carpogonium, then remaining passive, three secondary, actively initiating conjugation tubes which reach the primary and then become the seat of the origin of the sporogenous threads (Figs. 4 and 8).

We may assume, I think, from the researches of Oltmanns (1898) and Yamanouchi (1907) that the diploid nucleus formed in the carpogonium is transferred to the primary auxiliary, where it divides, and from which in turn there is supplied, by means of the conjugation tubes, at least one diploid nucleus to each of the secondary auxiliary or placental cells. A large and conspicuous nucleus is very characteristic of these placental cells immediately after the conjugation has been set up.

From each of these three auxiliaries there now grows out what in time becomes a dense fascicle of filaments (gonimoblasts) ending in moniliform chains of carpospores to the number of several score to each fascicle. While these fascicles are forming, the fertile branch, or cystocarp as we may now call it, is distended outwards and upwards, becoming thereby, owing to the local pressure of the fascicles, somewhat 3-lobed, with the morphological apex, which has ceased to grow after fertilization, depressed between. During the course of this development the appendages of limited growth are gradually shed, remaining only at the base as a sort of involucre.

The maturer stage has been accurately figured by the Crouan brothers in the 'Florule du Finistère' (1867) (Fig. 10).

I may note here that Schmitz and Hauptfleisch, in their diagnosis of the Spyridieae, speak of the cystocarp as being at first 2-lobed, and later 3- or several-lobed. I have always found it 3-lobed.

The figure of the mature cystocarp given by Farlow (1879) and reproduced by Hauck (1885) requires explanation. It is represented as deeply 2-lobed, but as in one of the lobes an axial row of cells is shown, it does not correspond to one of the lobes of which I have spoken already, where of course there can be no such axial row of cells. The explanation is simple. I have frequently found arising from the stalk of a procarp-bearing branch, immediately below the point at which it takes on the formation of procarps, another such procarp-bearing branch. I have even found three such branches abutting against one another. In the case figured by Farlow two such branches have grown *pari passu*, and were each probably obscurely 3-lobed, though that cannot be made out in the figure. It is really a double or twin cystocarp and not a single 2-lobed cystocarp. Agardh (1897), in comparing his own figures of the cystocarp in the 'Florideernes Morphologi' (1879) with that of Farlow, declares that any one might suppose they belonged to different plants. In dealing with *S. biannulata*, a form near to *S. filamentosa*, Agardh found the cystocarp to be without lobes and subglobose. However, in *S. opposita*, a species supposed to be more remote from *S. filamentosa*, Harvey (1858) found a 3-lobed cystocarp and three 'nuclei', i. e. fascicles of carpospores.

Again, from Farlow's figure it might be supposed that the whole axial row of cells becomes sporogenous. As I have said, I have never found more than one axial joint concerned with spore-production, and then the sporogenous fascicles do not arise directly from the central cell, but from the three placental cells which I have described as secondary auxiliaries.

It follows from what has been said that the sporogenous threads are wholly distinct from the sterile threads within the embrace of which they are contained during the maturation of the carpospores. I cannot but regard the Figs. 12 and 13 of Agardh in Pl. XVI of the 'Florideernes Morphologi', where sporogenous threads are shown in organic connexion with sterile threads, as due to errors of observation. It is true, as I have shown, that frequent anastomoses occur between cells of the sterile threads, but I have never seen an anastomosis of a cell of a sterile thread with one of a sporogenous thread. Agardh's figures of *S. biannulata* and *S. cupressina* in Continuatio IV of his 'Analecta algologica' (1897) leave the same impression of organic continuity of sterile and fertile threads, but in these foreign species at any rate he was probably working with difficult dried material.

With regard to the whorled branches derived from the sterile central cells, it has to be noticed that a curious anastomosis occurs by which the second cells of the lateral branches above grow downwards and set up an anastomosis with processes which grow upward from the whorl of branches below the fertile joint. The derivatives of the fertile central cell do not thus share in the anastomosis, but, owing to the arching outwards of the anastomosing threads, are left isolated in a central spherical space containing the fertile central cell and its derivatives (Fig. 8).

The walls of the cells of the maturing cystocarp are exceptionally mucilaginous and swell up readily on treatment with suitable reagents.

The surface of the cystocarp, or pericarp, consists of the terminal cells of the sterile branches. A tenacious pellicle comparable with the cuticle of subaerial plants delimits the whole. The cystocarp during maturation gradually changes from being cylindrical to ellipsoidal with an apical tip,

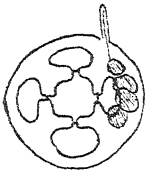

Fig. 5.

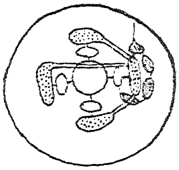

Fig. 6.

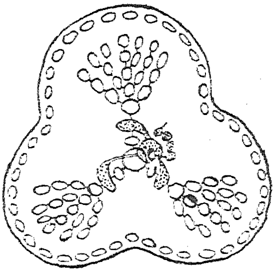

Fig. 7.

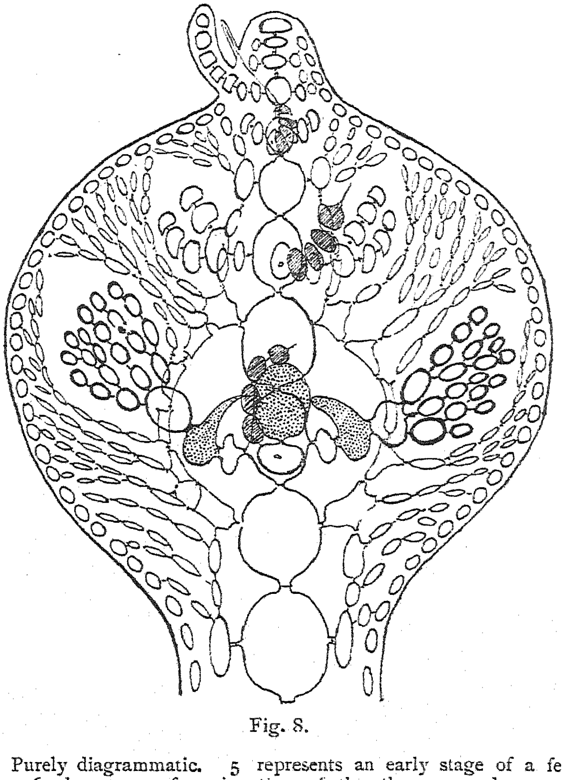

Fig. 8.

FIGS. 5, 6, 7. Purely diagrammatic. 5 represents an early stage of a fertile joint, corresponding to Fig. 3; 6, the stage of conjugation of the three secondary auxiliaries with the primary, corresponding to Fig. 4; 7, the stage when the gonimoblast fascicles are developing from the secondary auxiliaries, corresponding to Fig. 8. Markings as before.

FIG. 8. A more advanced stage, where the gonimoblast filaments have become a conspicuous feature. The conjugation tube on the left has disengaged itself from the primary auxiliary. The procarp-bearing branches above are developing vegetatively; no conjugation tubes are shown. They are recognizable, however, owing to their late development among the branches of the sterile joints above and below. × 350 *ca.*

the centre of distension being the fertile joint. Later the three swelling lobes bulge outward and upward, immersing the organic apex in a depression between the lobes (Fig. 10). There is no stomium, the carpospores being set free by the rupture of the surface pellicle and the dissolution of the contents. I am inclined to think that the carpospores escape in three dense fascicles, from which the individual spores escape later one by one.

With regard to the derivatives of a fertile central cell, where no fertilization takes place, or, if it does take place, where no further development ensues, I believe that the pericentral cells revert to the vegetative condition, and, growing out sterile branches, share in the formation of the cystocarp (Fig. 8). As, however, they do so much later than the corresponding cells above and below, they are distinguishable for a while and seem to promise the formation of fascicles of carpospores, but, as I have said, I have never found more than three such fascicles in a mature cystocarp (Fig. 7).

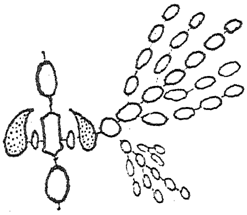

Fig. 9.

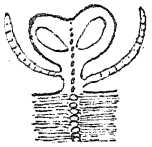

Fig. 10.

FIG. 9. Showing a small-celled branch arising from a secondary auxiliary, probably not a gonimoblast fascicle, but a sterile derivative of the auxiliary, which arose prior to conjugation and was thrust aside by the more vigorous gonimoblast fascicle. × 300.

FIG. 10. Vertical section of ripe cystocarp adapted from Figs. 4 and 10b, Pl. XV, of Crouan's 'Florule du Finistère'.

I have seen some evidence that, in the case of a fertilized joint, conjugation of the secondary auxiliaries with the primary auxiliary takes place, in some instances, after these secondaries have already begun to branch in a vegetative way. After conjugation, however, they branch much more vigorously, and the sterile products which they had produced before are thrust aside (Fig. 9). The new branches which Agardh describes as growing from the base of the fascicles of sporogenous filaments are probably such sterile products, for it seems to me that the carpospores for the most part mature together, and not in a continuous crop or successive crops, as Agardh and Bornet supposed.

To consider now the taxonomic position of *Spyridia* in the additional light here thrown upon the structure and development of the cystocarp.

In the first place, if emphasis is to be laid on the fact that the secondary auxiliaries send out processes to reach the primary auxiliary, instead of the reverse process, viz. the production of meta-ooblastemata by the first auxiliary, then it is impossible to find a place for *Spyridia* at all in the existing schemes of classification, for no such process has hitherto been observed among Florideae, as far as I am aware.

The object sought is no doubt the transference of diploid nuclei from the first auxiliary to the three of the second order, or placental cells, and the processes emitted by the latter are no doubt of the nature of haustoria. It is difficult to conceive of the protoplasmic movements involved in the process it is true, but as it is only after this suctorial process is completed that the placental cells can give rise to carpospores, it is certain from all analogy that diploid nuclei reach the placental cells. Let us assume that what happens is only the reversal of the process of the formation of meta-ooblastemata. If cells having a mutual chemical attraction lie sufficiently close together, it is conceivable that either or both may initiate a conjugating process. The varying modes in which zygospore-formation may occur among Conjugatae suggest such possibilities. *Spyridia* would seem to have stereotyped the production of the tubes by the placental cells. Where now on this assumption is *Spyridia* to be placed?

On the ground of structure it is natural to look for an alliance with Ceramiaceae. Agardh, however, on wider grounds, rather favoured the alliance with Rhodomelaceae. I have shown that its cortication is unlike that of *Ceramium*, much less *Polysiphonia*. And from what is now disclosed as to the intimate structure of the cystocarp, it is clear that it has no near relationship to either, or indeed to any of the families of the Rhodymeniales. Like them it has a fixed procarp with an auxiliary formed after fertilization, but unlike them it has also secondary auxiliaries which are seats of spore-formation.

Distinct groups of carpospores resulting from one act of fertilization occur both among Cryptonemiales and Gigartinales (using the latter term in the sense in which Oltmanns uses it in 'Die Algen' and not in the Schmitz-Hauptfleisch sense). In the latter, however, there is a well-defined procarp such as is found in *Spyridia*, and on that and other grounds it is among Gigartinales that I would look for an alliance for *Spyridia*.

Agardh at different times compared *Spyridia* with *Ceramium*, *Polysiphonia*, *Areschougia*, and *Wrangelia*. I have spoken of the relationship to the first two genera. *Areschougia* is a genus of the Rhodophyllidaceae, an order of Gigartinales, but it is difficult, judging from Agardh's figures of the cystocarp, to see the likeness with *Spyridia*.

With regard to *Wrangelia* we know, as the result of Zerlang's (1889) researches, following those of Bornet (1880), that the result of the fertilization of one procarp is communicated to the basal cell of other branches of the same whorl and ultimately similar cells of other whorls above and below. It is not inconceivable that *Spyridia* may be a highly specialized condition of what is diffuse in *Wrangelia*. Instead of producing procarps one to each whorl as in *Wrangelia*, let us suppose the procarp production is confined to alternate whorls. Instead of finding auxiliaries in several whorls, let us suppose them reduced to three in the same whorl. Instead of spores arising as it were from a kind of hymenium as in *Wrangelia*, let us suppose them localized in three fascicles. There is no great difficulty in finding resemblances in the vegetative characters of *Wrangelia* and *Spyridia*.

It would be tedious to examine the other cases of a discrete cystocarp resulting from one act of fertilization. I cannot find in any of them a close resemblance with *Spyridia* with its three definite auxiliaries located in the same whorl of branches as the procarp.

It is, I admit, a far cry from *Spyridia* to *Wrangelia*, but in default of a better suggestion I would provisionally place Spyridiaceae as a distinct order alongside Wrangeliaceae in the cohort Gigartinales (*sensu* Oltmanns).

SUMMARY.

1. *Spyridia*, from its wide geographical distribution, would seem to be an old and well-established stock.
2. The mode of cortication of its main axes does not suggest a close affinity with *Ceramium* or *Polysiphonia*.
3. The procarps occur on adventitious branches of unlimited growth, whose elongation, however, is arrested on the fertilization of a procarp.
4. The procarps arise singly on alternate joints of the main axis, and consist of a 4-celled carpogonial branch arising from a pericentral cell. Only one such procarp on a whole axis gives rise to spore-production.
5. An auxiliary cell arises after fertilization, from the pericentral cell, with which the carpogonium fuses, but which is not the seat of spore-production.
6. The second cells of the three other branches on the same joint act as secondary auxiliaries, and send forth haustoria which penetrate to the first auxiliary, and afterwards become seats of copious spore-production.
7. The cystocarp is, when mature, 3-lobed, corresponding to the three fascicles of carpospores.
8. *Spyridia* has no near affinity with Ceramiaceae or any Rhodymeniales, and it is suggested that provisionally it should be given ordinal position alongside Wrangeliaceae in the cohort Gigartinales (*sensu* Oltmanns).

I am grateful to my successor, Professor Thoday, for his courtesy in affording me accommodation in his laboratory for the purpose of continuing this investigation.

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The Staling of Fungal Cultures.

I. General and Chemical Investigation of Staling by Fusarium.

BY

CLARA A. PRATT, M.A.

(*Department of Plant Physiology and Pathology, Imperial College of Science and Technology, London.*)

With one Figure in the Text.

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INTRODUCTION.

IT is known that the rate of growth of a fungus on a limited quantity of artificial medium diminishes gradually and finally ceases. The culture is then said to have become 'stale'. Now this phenomenon has recently been considered to be independent of the reduction in the amount of nutrient present, but to be due to chemical changes in the medium brought about by the metabolism of the fungus. It was desirable to determine how far these conclusions are justifiable, and to investigate the nature of the metabolic products of fungi grown in liquid culture—in particular the effect of those products on the growth of fungi.

'Staling' is not only of importance in relation to the proper conditions for the artificial culture of fungi in the laboratory, but it must also play an important part in controlling the rate of progress of a parasite in the infected tissue of a host plant. It is thus one of the fundamental problems of plant pathology.

HISTORICAL.

While there is a large literature on the chemical products of certain fungi, notably of *Aspergillus niger* and *Citromyces* (*vide* Wehmer in Lafar's 'Handbuch', 1907, Bd. iv, p. 239), there has been less work on the effects of the products of their metabolism on the growth of fungi. Earlier workers confined their observations chiefly to yeasts and bacteria. Of these, the pioneer was Pasteur, who showed that the growth of yeast was retarded by the accumulation of the alcohol which it produced. Similarly, lactic and butyric acid were shown to stop the growth of the bacteria which produced them.

Duclaux (1900) propounded a general law that the medium in which an organism grows becomes more and more unfavourable for growth from generation to generation, i. e. growth in it of a fungus unfits the medium for subsequent growth of a fresh inoculation of the same fungus.

An important early research on the effects on the growth of moulds of the products of their own metabolism is that of Nikitinsky (1904). An account of this work is given by Boyle (1924), so it will suffice here to say that cessation of growth can, in each of the cases which he describes, be directly attributed to the chemical nature of part of the food supply. With different sources of nitrogen the medium became acid or alkaline, according as the basic or the acid radical was consumed by the fungus.

Eijkman (1904) demonstrated that thermolabile staling substances were produced in bacterial cultures and were the chief agents in causing stoppage of growth. Heating the gelatine medium rendered it fit for further growth of bacteria. The effect was not due, according to him, to a volatile substance, as was shown by heating the medium in a sealed tube. Growth in heated cultures was not, however, so good as that in fresh tubes, especially when the cultures were old. This may be due, he suggests, to exhaustion of food or to the presence of thermostable substances.

A criticism of Eijkman's work was published by Manteufel (1907), who declares that the sole factor is exhaustion of food. Exhaustion of nutrient, he says, is intensified by a process of 'deutilizing' the nutrient by the action of the metabolic products of the organism in question (*B. coli*). This, of course, largely destroys the value of his criticism, as it really amounts to an admission of the important effect of these products. Manteufel considered that the effect of heating the stale liquid is due to the killing of the organisms present and the liberation of their cell-contents as food. He did not determine whether any food remained, nor did he try the effect of adding more nutrient, both of which are vital points in the establishment of his hypothesis.

However, it is clear from the work of Eijkman and of Manteufel that heating does not completely restore the nutritive value of a medium in which bacteria have grown.

Küster (1908) states that the growth of fungi, such as *Aspergillus*, *Botrytis cinerea*, *Mucor mucedo*, brings about great changes in the medium, the assimilation of the food substances resulting in the production of acidity or alkalinity and alteration in osmotic pressure. His experiments showed that, in many cases, a stale medium which has been boiled then allows more or less of germination and growth of fungus spores (sown in drops on slides). Even after the addition of more nutrient, the difference between the boiled and the unboiled medium was marked. The fungus which caused the staling and the new one need not, he found, be the same; there is, apparently, no specificity of staling substances.

Lutz (1909), experimenting with *Aspergillus niger*, *Botrytis cinerea*, *Cladosporium herbarum*, *Fusarium solani*, *Mucor mucedo*, *Penicillium glaucum*, and *Rhizopus nigricans*, came to the same conclusions. He points out that the thermolabile nature of the staling substances suggests that they are enzyme-like. He was unable to determine the chemical nature of the compounds, but showed that, in some cases, 'passage through a porcelain filter removed the enzyme'. Exposure to direct sunlight caused deactivation of these substances.

Ritter (1909) grew different moulds on glucose + salts + an ammonium salt (phosphate, sulphate, nitrate, chloride), and determined the dry weight of the mycelium and the acidity of the medium. He concluded that capacity to assimilate ammonia from its inorganic salts is inverse to the strength of the acid, and in direct relation to the capacity of the particular fungus to resist acid concentration. He places the fungi in order of tolerance to acids, thus: *Aspergillus niger*, *Rhizopus nigricans*, *Mucor racemosus*, *Mucor spinosus*, *Thamnidium elegans*, *Mucor mucedo*.

Ritter (1911), found development of alkalinity on potassium nitrate by *Mucor*, *Thamnidium*, and *Rhizopus*. This alkalinity is said to be due to the formation of potassium carbonate; by using calcium nitrate instead of potassium nitrate he found that the medium remained neutral or just acid.

Ritter (1914) grew *Aspergillus niger* on a synthetic medium in which the concentration of ammonium nitrate was varied—(*a*) 0·4 per cent., (*b*) 0·8 per cent., (*c*) 1·6 per cent. The highest dry weight of mycelium was obtained in (*a*) and the least in (*c*). This was said to be due to the inhibiting action of the acid liberated; in (*b*) and (*c*) a considerable amount of nitric acid was liberated. In (*a*) a certain amount of free oxalic acid was formed.

A similar effect had been observed previously by Tanret (1896), who grew *Aspergillus niger* on Raulin's solution with increased ammonium nitrate, when nitric acid was set free and no oxalic acid obtained.

Medisch (1910), in the course of a study of the conditions for the formation of colour by *Hypocrea rufa* grown on a synthetic medium, found that ammonium salts, especially of inorganic acids, prevented it, and that these cultures became acid. When nitrogen was supplied in the form of a nitrate of an alkali, or an alkaline earth, the culture became slightly alkaline and the yellow colour developed. The addition of acid prevented the appearance of the yellow colour.

Oxalates were demonstrable in potassium nitrate cultures of *Hypocrea* which had become alkaline. When ammonium chloride and potassium nitrate were offered together, only the ammonium chloride was used; in the case of ammonium nitrate, NH_4 was used and nitric acid accumulated. If NH_4-inorganic and NH_4-organic acid were added together, only the latter was used.

Wehmer (1913) described the development of a toxic degree of acidity in cultures of *Penicillium glaucum* on sugar and ammonium sulphate, due to the using up of the ammonia and the liberation of free sulphuric acid which accumulates. A similar effect was not obtained with ammonium nitrate, as the nitric acid was also used up (a different result from those obtained by Ritter and others). These results were not obtained with *Aspergillus niger* grown under the same conditions.

Molliard (1912) demonstrated the hypertrophying effect on pea roots of the stale medium in which *Rhizobium radicola* had grown for ten days. He found that the effect was reduced if the stale liquid were diluted with the original culture solution, or if it were boiled.

Reed and Grisson (1915) found development of alkalinity in month-old cultures of *Glomerella rufomaculans* grown on Czapek's solution (where the nitrogen source is sodium nitrate). Autolysis had then set in. They attributed alkalinity to: (1) carbonates (estimated by the Fresenius-Claussen method), (2) ammonia, (3) organic bases (see Reed, 1914).

Boas (1918) showed the influence of the nitrogen source in metabolism. He grew *Aspergillus niger* with urea or biuret as the source of nitrogen. With urea there was a very slight development of acidity, since ammonia was also produced. With biuret there was a gradual rise of acidity, but growth was slow, as this compound is a poor nutrient. The growth of *Cladosporium* in urea solutions was stopped by ammonia. The cessation of growth occurred later if ammonium salts of inorganic acids were the nitrogen source, acid poisoning occurring in this case. *Aspergillus* escapes rapid destruction on urea by production of oxalic acid.

The same author (Boas, 1919) obtained similar results with *Aspergillus niger* on a medium consisting of 5 per cent. maltose, 2 per cent. urea, 0·25 per cent. ammonium sulphate, 0·15 per cent. magnesium sulphate. The fungus was killed by ammonia. So also was *Cladosporium*, while *Botrytis cinerea* and *Oidium* are not killed under these conditions.

W. Brown (1923) studied the staling of *Sphaeropsis malorum* and *Fusarium* sp. in Petri dish cultures on potato agar. He finds that the relative amount of staling can be modified by varying the depth of the medium, and particularly by removing two volatile products of the metabolism of the fungus, namely, carbon dioxide and ammonia. Ammonia, the factor of staling particularly dealt with in his paper, is not, he says, the only staling factor, or the only alkaline one. If the ammonia is driven off from a medium on which either of these fungi have grown, the medium is still alkaline. Thus, a fixed alkali is formed, especially by *Fusarium*. He correlates the fact, reported in an earlier paper (1922), that acceleration of growth of *Sphaeropsis* and *Fusarium* occurs in increased percentage of carbon dioxide with their production of alkalinity in staling. The carbon dioxide delays staling by neutralizing the active products.

Boyle (1924), working in this college 1920–1, studied the growth of *Fusarium* in liquid culture in Richards' solution, potato extract, and apple extract. The first two media became alkaline, but apple extract became only slightly less acid. He found that Richards' solution is the most suitable for studying the problem of staling. He says that in the early stages of staling the inhibiting action of a staled medium on germination and growth can be partly removed by boiling. Restoration of the pH value approximately to that of the control also partly removes the growth-retarding effect. Boiling and corrections of pH value may have, he says, 'cumulative effect' when separately they have none. He shows that some of the toxic properties of the solution are removed by forcing it through a collodion membrane, which suggests that some, at any rate, of the staling substances are colloidal in nature.

Boyle treated the stale medium with absolute alcohol, with the idea that the staling principle might be enzymatic, but obtained no conclusive results.

J. F. Clark (1902) and Fulton (1908) demonstrated the deleterious action of the products of metabolism of fungi from an unusual point of view. The results obtained by both workers may be summed up in the words of Fulton: 'All of the fungi tested show a tendency to turn from a region in which hyphae of the same kind are growing, toward one destitute of hyphae, or in which the hyphae are less abundant. . . . This may be regarded as a negative reaction to stimuli from chemical substances which owe their origin in some way to the growing fungus.'

Graves (1916), following the work of Clark and of Fulton, found that the germ-tubes of *Rhizopus nigricans* showed a marked negative chemotropic reaction to a medium in which the same fungus had been growing for some time: this is, he suggests, a reaction of the fungus to its 'staling substance'. These substances appeared to be either thermolabile or volatile, for he showed that boiling a solution containing them markedly reduced their negative chemotropic influence.

A. J. Brown (1905), working with yeasts, came to the conclusion that neither carbon dioxide nor the non-gaseous products of cell metabolism are the determining causes of the arrest of cell reproduction. The decrease in velocity of fall in the rate of yeast reproduction as the number of cells increases indicates that the arresting influence is not the accumulation of the products of metabolism, for, presumably, in such a case the velocity of fall in rate would increase, but rather is due to exhaustion of some influence which produces a stimulating effect. This theory is particularly interesting in that it is contrary to the more normal view, and, in fact, at first sight lends support to the theory that exhaustion of the medium is of primary importance. But the controlling influence, as shown by Brown's experiments, is not associated with nutrition. Maximum growth is independent of amount of food; the arrest of cell reproduction is due to lack of oxygen. In the presence of a continuous supply of oxygen regulation of the reproductive functions of the yeast cell then appears, he says, to be determined by the food supply.

H. T. Brown (1914) states that, within certain limits of oxygen supply, the maximal reproduction of yeasts is strictly proportional to the initial amount of the oxygen dissolved in the liquid.

The later work of Slator (1918) on the growth of yeasts on a nutrient medium indicates that there are five phases of growth: (1) lag phase; (2) logarithmic phase; (3) retarded phase; (4) yeast crop; (5) death of yeast cells. In the retarded growth phase, which is comparable to the 'staled phase', the yeast cells are first influenced by carbon dioxide and later by lack of oxygen. Although alcohol acts as a retarding agent, growth usually stops before the alcohol is sufficient to exert much influence. The final cessation of growth is due, usually, to lack of oxygen.

It would appear, then, that 'staling' of yeasts is of a particular type, for the oxygen supply acts in this case as a limiting factor.

In recent years emphasis has been laid on the importance of the hydrogen-ion concentration as a factor influencing physiological processes. Webb (1919 and 1921), who gives a comprehensive account of the literature on the subject, studied the relation of spore germination and hydrogen-ion concentration in the cases of *Aspergillus niger*, *Penicillium*, *Botrytis*, &c. The influence of the pH value depends, he says, on the organism; the hydroxyl ions appear to be relatively more toxic to the spores studied than the hydrogen ions.

Zeller, Schmitz, and Duggar (1919) grew wood-destroying fungi on liquid media and found that the acidity of the medium was normally increased. They decide that the hydroxyl-ion concentration does not appear to be a limiting factor in growth.

Zeller and Schmitz (1919) grew mixed cultures of wood-destroying fungi on plates, and observed any inhibition or stimulation of growth, with a view to determine whether such might not be the result of depletion of carbohydrates in the medium or a change in hydrogen-ion concentration. They came to the general conclusion that neither of these was the limiting factor for growth.

Karrer (1921), in a study of the effect of hydrogen-ion concentration on the accumulation and activation of amylase produced by certain fungi, states that the accumulation of amylase by *Fusarium* decreased gradually as a more alkaline medium was used, this decrease not being coincident with a reduction in the amount of growth. An increase in accumulation occurred in the intra- and extra-cellular amylase of *Colletotrichum Gossypii* as the nutrient solution used was less acid.[1]

EXPERIMENTAL METHODS.

Throughout the work to be described the staling of *Fusarium* sp. on a liquid medium was studied. *Fusarium* was chosen because it is a comparatively rapid 'staler'—more rapid, for example, than *Botrytis cinerea*—and because it has no obvious peculiarities of metabolism, such as has *Aspergillus niger*, in which large quantities of oxalic acid are produced and the culture quickly becomes strongly acid. The peculiar metabolisms of *Aspergillus*, *Penicillium*, and the yeasts have been the subject of much work, and it appeared probable that more light might be thrown on the general question of 'staling' if what might be described as a 'normal' fungus were used.

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[1] This may afford an explanation of the effect of hydrogen-ion concentration—that is, its control over the secretion of enzymes.

Richards' solution was chosen as the standard medium. Its composition is as follows:

KNO_3	... 10 grm.	Cane sugar	... 50 grm.
KH_2PO_4	... 5 grm.	$FeCl_3$	... trace.
$MgSO_4$	... 2·5 grm.	Water	... 1000 c c.

It is preferable to use a synthetic medium of which the composition is definite and can be varied at will, rather than to use a plant extract (such as potato or apple), the composition of which is uncertain and may vary from time to time. Richards' solution was used in preference to any other synthetic medium for the reason that it contains no ammonium compound (or organic nitrogen compound, whence ammonia is easily derived) as the source of nitrogen. It was thus hoped to avoid the masking of other staling effects by that of free ammonia, which would probably be produced from these other sources of nitrogen.

The criterion of staleness adopted was the average length of the germ-tubes of spores of *Botrytis cinerea* sown in drops of the filtered medium in which the *Fusarium* had grown. Measurements with a micrometer eye-piece of the germ-tubes of unicellular *Botrytis* spores are more readily carried out than in the case of the multicellular spores of *Fusarium*, since in the former the limits of the spore are sharply defined and the tube is stouter. Moreover, it is found that spores of *Botrytis* are much more susceptible to the metabolic products than are spores of *Fusarium* itself: stray spores of *Fusarium* in the drops of *Fusarium*-staled medium often have quite long germ-tubes when the growth of *Botrytis* spores is entirely inhibited. Since Lutz (1909), for example, has found that 'the products have no specific action, in the sense that they also influence germination and growth of the spores of other fungi than those which produced them', it seems justifiable to consider experimental convenience in this respect.

Other workers—for instance, Webb (1919)—have used percentage germination as a measure of a retarding effect, but in the present work it was found that in cases where germination was 100 per cent. or practically so, there might be very wide variation in germ-tube length in different liquids. Thus differences may be revealed by measurement of germ-tubes which would not appear in determinations of percentage germination. Obviously, the present method is somewhat limited, in that small differences in average germ-tube length are of little value, since they are of the same order as the individual differences between germ-tubes in any one drop. However, though the figures obtained are not susceptible of treatment with mathematical exactness, it is quite safe to assume that comparatively large differences in average length, which are readily perceptible to the eye, have a real significance.

The fungus was grown in Erlenmeyer flasks (capacity 300 c.c.) selected with flat bottoms so as to give a uniform depth of liquid. Each flask contained 50 c.c. of the medium and was sterilized for 20 minutes at 120° C These were inoculated from a sterile pipette with equal amounts (1 or 2 c.c.) of a suspension in sterile water of *Fusarium* spores. The cultures were incubated at 20° C. The liquid soon became straw-coloured and finally brown. Young cultures had a pleasant fruity smell; older ones smelt rather musty and were found to have become alkaline. At suitable intervals the mycelium was filtered off and the filtered medium treated in different ways.

The stale liquid was tested by allowing spores of *Botrytis* to germinate in drops of it placed on grease-free slides. To about 2 c.c. of the liquid in a test-tube one drop of a heavy suspension of spores of *Botrytis cinerea* was added. The spores were obtained from cultures on potato mush agar, 10 to 14 days old, and the suspension filtered through muslin to remove fragments of hyphae and concentrated by centrifugalizing. Drops, of uniform size, of the inoculated liquid were delivered from a sterile pipette on to each slide. The slides were placed in large Petri dishes, lined with moist filter-paper in order to prevent drying up of the drops, and left in an incubator at 14·5° C. overnight, i. e. for about 17 hours. This time and temperature were found to give a convenient length of germ-tube in the control, the aim being to get approximately the same value for the control each time, so that the results of separate experiments might be more nearly comparable.

In each case 50 to 100 (generally 100) germ-tubes were measured with a micrometer eyepiece, and the values given are the mean of these.

Determinations of the hydrogen-ion concentration (pH) have been made according to the method of Clark and Lubs.

EXHAUSTION OF FOOD.

As a preliminary to investigation of the toxic substances of unknown chemical nature which are postulated as the cause of staling, it is essential that evidence be brought forward to show that staling is not due in the present case to mere lack of some nutrient.

This evidence will be discussed under three heads, (*a*), (*b*), and (*c*).

(*a*) *The effect of dilution of the stale medium*, with water and with Richards' solution, was first studied. Spores of *Botrytis cinerea* were sown in the stale liquid in dilutions of 1 in 1, 1 in 9, 1 in 49, and 1 in 99, in distilled water, and in Richards' solution (R.S.).

Table I shows that there is enough food left to give much better growth than that in the control (water), even if the residual food is greatly diluted. When considerable food is added, e. g. 1 part of R.S. to 1 part of stale medium (Table II), germination is still very feeble. From Tables I and II it is obvious that the staling principle can be diluted so that its effect is weakened, since stale solutions will give germinations if sufficiently diluted.

TABLE I.

Effect on Botrytis Growth of Dilution with Distilled Water.

	Stale.	1 *pt. stale,* 1 *pt. water.*	1 *pt. stale.* 9 *pts. water.*	1 *pt. stale,* 49 *pts. water.*	1 *pt. stale,* 99 *pts. water.*	*Water.*
A.	0	0	0·65	2·01	3·81	1·6
B.	0	0	1·02	2·08	5·1	1·6

A. = 40 days stale. B. = 30 days stale.

TABLE II.

Effect on Botrytis Growth of Dilution with Richards' Solution.

Stale.	1 *pt. stale,* 1 *pt. R.S.*	1 *pt. stale,* 9 *pts. R.S.*	1 *pt. stale,* 49 *pts. R.S.*	1 *pt. stale,* 99 *pts. R.S.*	*R.S.*
0·59	0·41	4·8	5·61	8·91	10·73

21 days stale. pH 6·6.

TABLE III.

(*b*) *The Effect of Dilution of Fresh Richards' Solution.*

	R.S.	*R.S.*/10.	*R.S.*/100.	*R.S.*/1000.
Botrytis growth	10·11	13·31	14·05	
	10·4	10·88	11·6	8·52

From Table III it is clear that the exhaustion of nutrient would have to be extreme to produce an effect at all comparable with that of the stale solution. Indeed growth in R.S./10 and R.S./100 is somewhat better than in the undiluted medium, and is only reduced a comparatively small amount in R.S./1,000. As a matter of fact growth in the absence of external nutrient—that is, in distilled water—is found (see Table I) to be quite appreciable. For a germination test of short duration (as overnight) the concentration of the nutrient over a wide range has very little effect.

(*c*) *Quantitative determination of sugars.* The concentration of these remaining in some very stale cultures was found. The filtered medium was in each case heated with hydrochloric acid on a water-bath for 30 minutes to invert the cane sugar, then neutralized with powdered sodium carbonate and the volume made up to 50 c.c. (that of the fresh solution in each flask). The total sugars were estimated in the usual way by reduction of Fehling's solution.

The results of four such estimations are given in Table IV, from which it will be seen that there is still sufficient sugar remaining to give good growth of *Botrytis* germ-tubes.

TABLE IV.

Estimations of Sucrose.

Age of Culture.	*Grm. Sucrose per Litre.*	*Percentage Sucrose.*
3 months	0·59425	0·059
72 days	1·055	0·105
92 days	0·3894	0·0389
93 days	0·3262	0·0326

N.B.:

R.S. ... 5% sucrose
R.S./10 ... 0·5% ,,
R.S./100 ... 0·05% ,,
R.S./1000 ... 0·005% ,,

In these cases the solutions were made up to the volume of the fresh medium, whereas in that of the stale liquids tested with *Botrytis* this was not done. Now the volume of the medium becomes considerably reduced in the stale culture, so that the sugar in these solutions is actually present in higher concentrations than those suggested by the figures. Nevertheless, it must be pointed out that the figures may be somewhat too high owing to the fact that the determinations would include other reducing substances, besides sugars, which might be present.

Owing to the practical difficulty of estimating the salts in the staled medium, estimations of the amount of sugar remaining were taken to show, sufficiently closely, the exhaustion of the medium.

It is obvious, then, from the evidence put forward in (*a*), (*b*), and (*c*), that the staling effects, as measured by the germ-tube method, cannot be ascribed to reduction in the amount of nutrients present. The results indicate clearly that something inhibitory of germination is present in the stale liquid.

CHANGES IN THE MEDIUM, OTHER THAN DIMINUTION IN NUTRIENTS.

The parts played by reduction of food supply and metabolic products are indicated by the preceding considerations, and it is now proposed to study the second of these two factors—that is, those changes, other than diminution of nutrients, which are brought about in the medium by the growth of the fungus.

The main lines of attack may be briefly summarized:

I. Treatment of the stale medium with the object of restoring, wholly or in part, its power of inducing germination of fungus spores and growth of their germ-tubes, i. e. its 'germinative capacity'.

II. Chemical analysis, quantitative and qualitative, of the stale medium with a view to determine the nature of the metabolic products.

III. The effect on the growth of *Botrytis cinerea* of addition to the fresh medium of certain organic compounds.

I. TREATMENT OF THE STALED MEDIUM.

From a consideration of the results of other workers it appears that the 'staling' principle may be divided into three parts: (1) a substance which is thermolabile, (2) an effect which is the result of change in pH value, i. e. development of alkalinity, and (3) an effect which manifests itself after the removal of the other two.

It seemed profitable to bring forward further evidence to establish the existence of (1) and (2) before attempting to analyse the third.

TABLE V.

(i) *Effect of Boiling.*

Days stale.	*pH, &c.*	*Bot. growth stale.*	*Bot. growth stale; boiled.*	*Bot. growth R.S.*
4	4·6	6·59	7·6	7·45
5	4·2	5·1	5·8	—
6	5·0	4·95	8·0	7·58
7	5·0	4·23	5·63	—
8	4·8	3·32	7·5	—
19	6·4	0 + +	3·06	14·33
20	7·8	0	0 + +	16·02
22	8·0	0	0	16·93
27	8·4	0	0	16·4

The medium was boiled till reduced to about half bulk and then made up to the original volume. It will be seen that, when the medium is only a few (6 to 8) days stale, boiling brings the value for the *Botrytis* germ-tube length nearly up to that of the control, though the growth in the untreated medium is about half that of the control. In another series, even at 19 and 20 days old, boiling gave a perceptible improvement in the medium.

Some workers have considered that the staling principle is enzymatic or catalytic in nature, since it is partly deactivated by heating. The evidence is, on the whole, against this theory. The application by Boyle of the technique of enzyme precipitation with absolute alcohol gave, as already pointed out, inconclusive results. It is more probable, and certainly a more satisfactory working hypothesis, that the effect produced by heat is the result of the presence of volatile compounds in stale solutions.

Table V also shows the gradual reduction in the acidity of the cultures.

(ii) *The effect of restoration of the hydrogen-ion concentration* to that of the original Richards' solution (pH = 4·6) is shown below in Table VI.

The adjustment of the pH value was very carefully made. Boyle added hydrochloric acid, as it seemed possible, *a priori*, that the introduction of chlorine ions not already present in the medium might have some independent effect; in the experiments here described, restoration of the pH value was brought about by the addition of different acids. No appreciable difference was found between nitric, hydrochloric, and sulphuric acids. It was decided, nevertheless, to use nitric acid, rather than hydrochloric acid, for this purpose in subsequent experiments.

TABLE VI.

Days stale.	*Stale Bot. growth.*	*Stale pH.*	*Acid added.*	*Stale and acid Bot. growth.*	*Control R.S. (pH = 4·6).*
20	0·49	6·6	HNO_3		9·16
20	0·49	6·6	HCl	0·81	
20	0·49	6·6	H_2SO_4	1·07	
35	0	9·4	HNO_3	2·35	
35	0	9·4	HCl	2·65	
35	0	9·4	H_2SO_4	2·19	
21	0·56	6·4	HNO_3	1·65	7·23
21	0·56	6·4	HCl	1·65	

(iii) *The Combined Effect of Acidifying and Boiling on Botrytis Growth.*

When the stale medium was acidified and then boiled, it was found (Table VII, column 6) that the combined effect of the two together was greater than the sum of their separate effects, and that an unexplained residue of 'staleness' still remained.

TABLE VII.

Days stale.	*pH.*	*Stale.*	*Stale; boiled.*	*Stale; HNO_3 to 4·6.*	*Stale; HNO_3; boiled.*	*RS. control.*
19	6·4	0+ +	3·06	3·23	7·33	14·33
20	7·8	0	0+ +	3·3	7·03	16·02
22	8·0	0	0	3·72	5·98	16·93
27	8·4	0	0	3·3	11·53	16·4
30	8·0	0	0	4·24	7·55	13·58
49	8·8	0	0	1·88	4·20	8·75
50	9·0	0	0	4·77	6·95	10·30
51	9·0	0	0	4·71	6·75	8·65
52	9·0	0	0	4·75	8·02	11·6

(iv) *Extraction with Ether.*

Boyle made an unsuccessful attempt to extract staling substances with ether. He used the alkaline stale medium, in which, as is shown below, ether extraction has no effect. If, however, the medium be first acidified it has been found that ether treatment will restore to stale Richards' solution its germinative capacity.

Extraction with ether was carried out in a separating funnel. A measured volume of the aqueous layer was evaporated to half bulk, in order to drive off any ether, and the volume made up with distilled water.

Now, it seemed possible that the removal of staling substances from an alkaline medium might occur, but the fact would remain undetected unless the pH value were afterwards corrected. Accordingly, the alkaline medium was extracted with ether and then acidified (to pH = 4·6). *Botrytis* growth in this liquid and in the boiled (i. e. treatment comparable to evaporation) and then acidified stale medium was compared. There was no difference between these two (see Table VIII), showing that no toxic substance is extracted from the alkaline medium.

TABLE VIII.

Ether Treatment of Alkaline Medium.

Days stale.	*pH.*	*Stale ; Bot. growth.*	*Stale ; boiled ; acid ; Bot. growth.*	*Stale ; ether ; boiled ; acid.*	*R.S.*
35	7·0	2·64	5·09	4·91	7·74
121	9·4	0	5·23	5·39	7·74

When the stale medium was first acidified, ether treatment was found to be effective. *Botrytis* growth in the acidified, extracted with ether and boiled stale medium, was greater than in the acidified and boiled medium, yet, in the preliminary experiments, it never became as good as in the control.

Before using the fact of extraction by ether after acidification as the basis of any argument as to the nature of the metabolic products, it is essential to examine critically the question of treatment by ether. Apart from the extraction of some inhibiting substance, ether might have two effects. (1) Boiling with ether might introduce some stimulant which would account for the increased growth. (2) Some inhibiting substance might be introduced with the ether—that is to say, some impurity of the ether, since the ether itself is entirely driven off by the evaporation. This second effect might account for the fact that the *Botrytis* growth value was not brought up to that of the control by ether treatment in the preliminary experiments, when commercial ether had been used.

These points have been examined by comparing the effect of treatment with ether without separation, with that of ether treatment followed by separation. Purified ether was used in the experiments, the results of which are given in Table X.

The details of the experiments are as follows (the numbers refer to those in the first column of the table):

(1) *Botrytis* growth in stale medium.

(2) *Botrytis* growth in stale medium, boiled.

(3) *Botrytis* growth in stale medium, acidified with nitric acid to pH 4·6.

(4) *Botrytis* growth in stale medium, acidified and boiled.

(5) *Botrytis* growth in stale medium, acidified, extracted with ether, separated, evaporated to half-bulk, &c.

(6) *Botrytis* growth in stale medium, acidified, extracted with ether, not separated, the whole evaporated to half-bulk, &c.

TABLE IX.

Ether Extraction of Acidified Medium.

Days stale.	49	50	51	52
pH	8·8	9·0	9·0	9·0
(1)	0	0	0	0
(2)	0	0	0	0
(3)	1·88	4·77	4·71	4·75
(4)	4·20	6·95	6·75	8·02
(5)	7·38	10·71	9·98	11·5
(6)	4·7	6·86	—	8·86
B. g. R.S.	8·75	10·30	8·65	11·6

Progressive improvement in *Botrytis* growth was obtained as the result of treatments (3), (4), and (5). The growth in (5) is, in each case, as good as that of the control; the growth inhibitors, active after boiling and acidifying, have been completely removed by the ether. In (6), however, the growth is practically the same as in (4); that is, no stimulant has been added—the growth observed is that due to acidifying and boiling the medium alone—neither is an inhibitor introduced, for the growth is not lower than in (4), as it would then have been.

It was therefore concluded that some impurity of the ether used in the preliminary experiments had introduced an inhibiting effect, but that if pure ether were used the effects observed were attributable solely to extraction of staling substances by the ether. This conclusion is supported by the fact that treatment of fresh Richards' solution with ether, with or without separation, was found to leave its germinative capacity unreduced.

The fact that a growth-retarding factor is removed by the ether treatment after, but not before, acidification throws light on the nature of this factor. If we suppose growth-retarding organic bases to be present, they would in all likelihood be extracted by ether from the alkaline stale medium, but not from the acidified medium, in which they would be present as salts. This, however, does not fall in with the experimental findings. But if we suppose an organic acid to be present, the reverse would be expected, for the free acids are more likely to be dissolved together than are their salts. Hence these results point to the presence of organic acids as being responsible for the effects observed.

(v) *Oxidation.*

Experiments were undertaken, in order to determine whether treatment with an oxidizing agent would remove staleness from a used medium.

The oxidizing agent first used was hydrogen peroxide in the usual '20-volume' solution.

In the experiments an excess of hydrogen peroxide solution was added to the filtered stale medium. The mixture was shaken at intervals. The solution was then boiled for fifteen minutes with charcoal, to remove excess of hydrogen peroxide, filtered and made up to the original volume with distilled water poured through the charcoal on the filter. Examples of the *Botrytis* germ-tube lengths obtained in drop cultures of the treated medium are given in Table X.

TABLE X.

Effect of Treatment with Hydrogen Peroxide and Charcoal.

Days old.	*pH.*	*Stale.*	*Stale; boiled.*	*Stale; boiled with charcoal.*	*Stale; H_2O_2; boiled with charcoal.*	*R.S. control.*
28	4·6	0	0	13·68	9·92	14·54
29	4·6	0	2·30	11·56	10·66	14·97
35	4·6	0	0·72	8·22	7·98	11·05

The medium was Richards' solution with one-third concentration of asparagin. There was no better growth after treatment with hydrogen peroxide and boiling with charcoal than after boiling with charcoal alone. The general conclusion is that the effects observed are really due to adsorption on the charcoal.

Gaseous oxygen, bubbled through the liquid, proved ineffective in removing staleness from the medium.

(vi) *Adsorption of the Toxic Substances.*

Investigation of adsorption was suggested by the results of treatment with charcoal in the hydrogen peroxide experiments.

Search was therefore made for a more efficient absorbing agent, and attention was drawn to a medical preparation of colloidal kaolin—'Collosan'—which was said to be free from injurious matter, organic or inorganic.

Experiments were carried out with this kaolin (see Table XI).

TABLE XI.

Effect of Treatment with 'Collosan' on Botrytis Growth.

Days stale.	*Stale.*	*1*	*2*	*3*	*4*	*5*	*6*	*R.S.*
70	0	0	0	6·56	14·72	8·67	14·03	16·4
73	0	0	0	4·1	9·49	8·54	9·77	—
36	0	0	0	2·0	6·0	—	6·38	7·47
				4·0	12·02		12·76	14·94
140	0	0	0				10·65	10·13

Details of Treatment:

Column 1 ... Boiled.
„ 2 ... 'Collosan'.
„ 3 ... Acid.
Column 4 ... Acid, 'Collosan'.
„ 5 ... Acid, boiled.
„ 6 ... 'Collosan', acid.

Here, again, a very marked restoration of germinative capacity is produced—growth often very nearly approaching that of the control. It makes no difference whether the stale medium is acidified before or after kaolin treatment, but no effect is manifest until alkalinity is removed.

Shaking fresh Richards' solution with kaolin did not reduce or increase the subsequent growth in it; that is, neither an inhibiting nor a stimulating effect was introduced. Attempts to recover the staling substances absorbed on the kaolin have so far been unsuccessful.

II. CHEMICAL INVESTIGATION.

During the growth of *Fusarium* in Richards' solution, the original colourless liquid gradually becomes yellow and finally yellowish brown. In very stale cultures the medium is dark brown in colour. In the early stages the cultures smell sweetish and slightly alcoholic, but this smell is gradually lost and the older cultures have merely a somewhat musty odour. There is no distinct smell of ammonia.

(i) *Stale Medium.*

The brown colour of the stale medium and the presence of phosphates make qualitative analysis of it difficult and unsatisfactory. The following tests were applied:

(1) Fehling's solution, ammoniacal silver nitrate, and potassium permanganate were reduced, probably by the reducing sugars present.

(2) Ferric chloride tests for organic acids were unsuccessful, owing to the presence of phosphates and the brown colour of the liquid. Even after the removal of phosphates by magnesia mixture the brown colour remained, and the tests gave no results.

(3) The presence in the stale medium of organic acids or compounds of them was indicated by the production of an ester with a strong fruity smell on warming with concentrated sulphuric acid and alcohol.

(4) A positive iodoform test was obtained several times, indicating the presence of alcohols, or possibly of aldehydes or ketones.

(5) The presence of amines (carbylamine test) or of organic bases (murexide test) could not be shown.

(6) In spite of repeated efforts, using the method described by Raistrick and Clark, no conclusive proof could be obtained of the presence of oxalates.

(ii) *Distillate.*

In the hope of discovering the chemical nature of the thermolabile part of the staling principle, the stale medium from a few cultures was distilled and the distillates tested.

(1) Slight reduction of Fehling's and of ammoniacal silver nitrate was obtained.

(2) The iodoform test gave positive results, showing the presence of alcohols.

(3) Schiff's reagent for aldehydes gave negative results.

(4) Carbylamine test for amines gave negative results.

(5) Ammonia was tested for with Nessler's reagent. It was found that the first fraction distilled over gave a heavy orange precipitate, and so did the second. Subsequent fractions gave Nessler's test in diminishing degree.

An example may be given of one such distillation of stale medium, 57 days old, pH = 9·2:

Fraction (1) pH = 9·2. Heavy Nessler's precipitate.
(2) pH = 8·2. Good Nessler's precipitate.
(3) pH = 6·8. Slight Nessler's precipitate.
(4) pH = 6·4. Cloudy yellow colour.
(5) pH = 6·2. Only slight yellow colour with Nessler's solution.

Water was added to the residue, and the liquid again distilled until reduced to a small bulk. This was then made up to the original volume. The solution was more strongly alkaline than before (its pH = 10+), but gave no precipitate with Nessler's solution. The alkalinity of the residue cannot, therefore, be attributed to ammonia compounds.

When the acidified stale medium (pH = 4·6) was distilled, and the slightly acid distillate tested with concentrated sulphuric acid and alcohol, the fruity smell of an ester was obtained, showing that some volatile organic acids which had been liberated by the acidifying had come over with the distillate.

The alkaline residue left after distillation of the alkaline stale medium also gave this ester test.

(iii) *Ether Extract.*

The ethereal layer obtained from the extraction of several stale acidified cultures was evaporated. A small, sticky residue, with a smell like honey, was obtained and dissolved with some difficulty in a little water. With this solution an ester test could be obtained. The fruity smell seemed to be rather different from that obtained previously. The solution also reduced permanganate in the cold. This reduction may be due to the presence of sugars or of unsaturated organic acids.

Schiff's reagent did not show the presence of aldehydes.

(iv) *The Organic Acids formed by Fusarium.*

In order to obtain more definite evidence of the formation of organic acids by *Fusarium*, an attempt was made to isolate them in identifiable quantities.

The medium was filtered from twenty cultures of *Fusarium* on Richards' solution, three weeks old. About 750 c.c. of the straw-coloured liquid were obtained; it was still acid (pH = 5·6) and had a sweetish, slightly alcoholic smell.

The procedure was as follows:

(A) The liquid was made alkaline with sodium carbonate and distilled with steam, so that the volume of liquid in the distilling flask remained approximately constant. About 100 c.c. of distillate (A) were collected.

(B) The residue was allowed to cool somewhat, and then acidified with an excess of phosphoric acid. A slight precipitate was filtered off and the filtrate distilled with steam until about 1,000 c.c. of distillate (B) had been collected.

(C) The residue was evaporated to about 200 c.c., allowed to cool, and then extracted with ether for several hours, in order to recover any non-volatile acids.

The distillates were examined as follows:

Distillate (A).

The colourless distillate (A) was alkaline (pH = 8·8) and had a peculiar, rather malty, odour.

(1) Warming with concentrated sulphuric acid and a drop of acetic acid gave a fruity smell of an ester, indicating the presence of alcohols.

(2) Iodoform test: positive for alcohols.

(3) Nessler's solution gave an immediate heavy orange precipitate, showing the presence of ammonia.

(4) On heating with sodium hydroxide the solution turned yellow and somewhat cloudy, and gave off a pungent, rather sweetish odour. This suggested that an aldehyde might be present.

(5) Ammoniacal silver nitrate, potassium permanganate, and dilute Fehling's solution were slowly reduced.

(6) Schiff's reagent was slowly turned slightly pink. A blank test left for the same time showed no colour. This was repeated with the same result. The presence of traces of aldehydes was thus indicated.

(7) On addition of gallic acid and concentrated sulphuric acid no reaction for formaldehyde was obtained.

(8) A solution of resorcinol and concentrated sulphuric acid gave a slight brown ring and a slight white precipitate (cloudiness). This may indicate traces of formaldehyde.

(9) Finally, Schryver's test for formaldehyde was applied as follows: to 10 c.c. of the distillate were added 2 c.c. of freshly made dilute phenylhydrazine hydrochloric solution (approx. 1 per cent.), 1 c.c. of freshly made potassium ferricyanide solution, and 5 c.c. of concentrated hydrochloric acid. The solution became pinkish yellow, quite different in colour from the greenish yellow of a blank test. According to Schryver, a pink colour shows the presence of formaldehyde, so that it is probable that the distillate contained a trace of this compound.

It was concluded that the distillate contained ammonia, small amounts of alcohol, and possibly traces of aldehydes and formaldehyde.

Distillate (B).

This distillate was acid, but contained no phosphoric acid (ammonium molybdate test). The whole distillate was titrated with baryta solution, using phenolphthalein as the indicator, and evaporated to dryness on a water-bath. The yellowish residue was extracted with absolute alcohol, with frequent rubbing with a glass rod, and left overnight. The solution was filtered next day, when it was found that a good proportion of the residue was insoluble in alcohol.

The alcoholic solution was evaporated to dryness in several watch-glasses. Small white residues were obtained and tested.

(*a*) When the residue was heated with a drop of concentrated sulphuric acid, a distinct cheesy smell was obtained, resembling that of valeric acid and not quite so rancid as that of butyric acid.

(*b*) When the residue was heated with two drops of absolute alcohol and one drop of concentrated sulphuric acid, a fruity smell of an ester was obtained: the smell resembled that of highly scented apples, and was not quite so sweet as that of the pine-apple. The presence of valeric acid, and possibly butyric acid, was thus indicated.

The residue, insoluble in alcohol, was dissolved in hot water and filtered free from a slight insoluble precipitate, presumably of barium carbonate. A clear yellowish solution was obtained. To part of the solution an excess of dilute sulphuric acid was added, and the free acids distilled off; a colourless solution, with a slightly cheesy smell, was obtained. This solution and the solution of barium salts were tested as follows:

(1) One drop of ferric chloride was added to 3 c.c.; a reddish orange colour was obtained with both; the blank was nearly colourless. The presence of fatty acids was thus indicated.

(2) Silver nitrate gave a slight cloudiness; when a drop of ammonia was added, the solution at once darkened till it became quite grey; a trace of formic acid was therefore suspected.

(3) Mercuric chloride gave no precipitate, so that the presence of formic acid was not confirmed.

(4) Warming with alcohol and concentrated sulphuric acid gave a strong fruity smell of an ester.

The remainder of the solution of barium salts was evaporated to dryness; it yielded only a small yellow residue.

The whole procedure was repeated with the stale medium from twenty more cultures (thirty days old, pH = 7·0) with the object of obtaining a sufficient amount of the barium salts for quantitative analysis. The filtered solution of the alcohol-insoluble barium salts was evaporated to dryness on a water-bath. The yellowish shining residue was purified by redistillation from strong sulphuric acid. The small distillate had a strong smell of acetic or propionic acids. It was neutralized with baryta solution, and filtered free from a small amount of barium sulphate. The solution was evaporated to dryness, and the residue transferred to a weighing bottle and dried at 130° C. A weighed amount of this dry salt was dissolved and treated with hot dilute sulphuric acid. The weight of barium sulphate was determined in the usual way. 0·0626 grm. of the salt yielded 0·0556 grm. of barium sulphate, i.e. 88·8 per cent. barium sulphate.

Now the barium salts of fatty acids yield theoretically the following percentages of barium sulphate:

Barium formate	119·47	per cent.	barium	sulphate.
,, acetate	91·37	,,	,,	,,
,, propionate	82·13	,,	,,	,,
,, butyrate	74·91	,,	,,	,,
,, valerate	68·73	,,	,,	,,
,, caproate	63·48	,,	,,	,,

The theoretical percentages, 82·13 and 91·37, for barium propionate and acetate are nearest to the experimental value 68·8 per cent. It seems probable, however, that the salt analysed consisted of a mixture of the barium salts of several of the lower fatty acids.[1]

The remainder of the dry barium salt was tested:

(*a*) To the dry salt in a test-tube, two or three drops of absolute alcohol and one drop of concentrated sulphuric acid were added and the mixture was warmed. A very strong, fragrant smell of an ester was obtained. The smell did not exactly resemble that of either ethyl acetate, propionate, butyrate, or valerate, with which it was compared. It seemed to be a mixture of these smells.

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[1] The proportions in which any two of these could be present may be calculated thus:

If x = percentage of barium salt of lower fatty acid,

P = barium sulphate yielded by mixed salt,

B = percentage of barium sulphate theoretically obtainable from the pure salt of the lower fatty acid,

b = percentage of barium sulphate theoretically obtainable from the pure salt of the higher fatty acid,

then $Bx = 100P + bx - 100b$.

Whence it is found that, assuming that they alone were present, the mixed salt would consist of 72·19 per cent. barium acetate and 27·81 per cent. barium propionate, or, similarly, of 88·65 per cent. barium acetate and 11·35 per cent. barium valerate.

(*b*) A portion of the dry salt was dissolved in a very little water and a drop of very dilute ferric chloride solution added. A deep reddish orange colour and a slight precipitate were obtained, showing the presence of acetate and propionate, and probably of salts of other fatty acids.

Ether Extract (C).

The ether extract was evaporated to dryness and the brown sticky residue dissolved in a little water. The solution was tested for lactic acid by the method of Uffelmann. A reagent was prepared by mixing 10 c.c. of a 4 per cent. solution of phenol with 20 c.c. of water and adding one drop of a solution of ferric chloride. This violet liquid is said to be turned yellow by a solution of lactic acid containing only 1 part in 10,000. The aqueous solution tested turned the reagent yellow. A solution of phosphoric acid or of mineral acids merely decolorized the reagent. The presence of lactic acid in the stale medium was therefore inferred. Other non-volatile acids could not be demonstrated.

Thus very definite evidence has been obtained of the production of fatty acids by the growth of *Fusarium* on Richards' solution. It is more difficult to determine exactly which of these acids are formed and in what proportions. However, the presence of acetic and propionic (which are practically indistinguishable), butyric, valeric, and lactic acids has been indicated.

(v) *The Crystals in the Stale Medium.*

A constant feature of stale cultures of *Fusarium* in Richards' solution, not hitherto described, is the presence of long, more or less needle-shaped crystals, free in the liquid and embedded in the fungus weft. These crystals were picked out with forceps and freed mechanically from as much as possible of the mycelium. Tests showed that the crystals were insoluble in water; they became white and opaque when boiled in water, but retained their shape. They were insoluble in alcohol, but soluble in dilute hydrochloric acid. The crystals were therefore dissolved in dilute hydrochloric acid and filtered to remove any pieces of mycelium. This solution was tested.

1. Nessler's solution gave an orange precipitate showing that an *ammonium* salt was present. A piece of mycelium considerably larger than any which might possibly be adhering to the crystals gave only an exceedingly slight precipitate with Nessler's.

2. Ferrous sulphate and concentrated sulphuric acid gave no brown ring, i. e. nitrate absent.

3. Barium chloride gave no precipitate, i. e. sulphate absent.

4. Ammonium molybdate, to which a drop of the solution was added, gave a heavy yellow precipitate, indicating the presence of a *phosphate*.

5. Ammonia gave a white precipitate of small star-shaped crystals (seen under the microscope).

6. On adding a solution of sodium hydroxide to the acid solution of the crystals a white gelatinous precipitate, not soluble in excess of the reagent, was obtained. This indicates the probable presence of magnesium.

The conclusion was then arrived at that the crystals consisted of magnesium ammonium phosphate, $Mg(NH_4)PO_4, 6H_2O$. This compound is practically insoluble in water and in ammonia, but is soluble in dilute mineral acids.

This conclusion was confirmed by preparing magnesium ammonium phosphate artificially thus: to a solution of magnesium sulphate was added ammonium chloride, ammonia, and then ammonium phosphate. A crystalline precipitate was at once obtained. This was examined under the microscope and found to consist of star-shaped crystals exactly resembling those previously obtained by the addition of ammonia to the acid solutions of crystals from the cultures. The addition of sodium hydroxide and sodium phosphate to magnesium sulphate did not result in the formation of these crystals: the precipitate was gelatinous and amorphous.

Thus the presence of ammonia is essential for the formation of the crystals. There is no ammonia originally present in Richards' solution: it must therefore be formed by the fungus. The ammonia which is fixed in an insoluble salt cannot be held responsible for any part of the staling.

(vi) *Estimations of Ammonia.*

A few rough estimations of free ammonia in the stale medium were carried out.

25 c.c. of stale medium were distilled into 25 c.c. of a standard (approx. N/5) hydrochloric acid solution. The condenser was washed out with distilled water and the washings and the acid made up to 100 c.c. 25 c.c. portions of this liquid were titrated with standard (approx. N/5) sodium hydroxide solution. Hence the amount of ammonia, expressed in c.c. N/10 solution per 100 c.c. stale medium, was calculated (see Table XII). The mean (2·43) of the figures obtained for the acid medium (0, 9, 10, 28 days) was taken to represent the experimental error and was subtracted from the values for the alkaline medium.

Thus, little free ammonia is present in old, alkaline cultures. It remains to determine what effect on *Botrytis* growth addition of free ammonia in these small amounts would have.

However, ammonia is actually produced in fairly large amounts, as is shown by the formation of the crystals of magnesium ammonium phosphate. Thus the ammonia—a potential staling substance—formed by *Fusarium* growing on Richards' solution, is nearly all removed from the sphere of action as an insoluble salt.

TABLE XII.

Age in days.	*pH.*	*Percentage N/10 ammonia.*	*Percentage N/10 ammonia, less experimental error.*
0	4·6	1·2	
9	4·6	3·00	
10	3·8	3·00	
28	7·0	2·52	
78	9·0	13·76	11·33
79	9·0	8·36	5·93
98	8.8	7·92	5·47

III. THE EFFECT ON THE GROWTH OF *BOTRYTIS CINEREA* OF CERTAIN ORGANIC COMPOUNDS.

Attempts have been made to discover what part those metabolic products which have been demonstrated chemically could play in producing the effects observed in a stale medium. It has been shown that boiling will, in the early stages of staling, give increased germinative capacity. Could this be accounted for by the removal of alcohols or aldehydes? Are the growth-retarding properties of organic acids great enough to justify the theory, suggested by the results of ether treatment, that they are the inhibitors which are effective in the acidified stale medium?

Answers to these questions were sought by adding these compounds in different concentrations to fresh Richards' solution and comparing the growth of *Botrytis* germ-tubes in drops of these liquids.

The effects produced by relatively large amounts of ethyl alcohol, butyl alcohol, and acetaldehyde are shown in Table XIII.

TABLE XIII.

Amount added.	*Approximate proportion in R.S.*	*Ethyl alcohol.*	*Butyl alcohol.*	*Acetaldehyde.*
5 c.c. R.S. + 1 c.c.	1 in 6	11·70	0	0
5 c.c. R.S. + 0·5 c.c.	1 in 11	as control	0	0 +
5 c.c. R.S. + 0·25 c.c.	1 in 21	,,	6·90	1·62
5 c.c. R.S. + 0·125 c.c.	1 in 41	,,	8·61	6·20
R.S. control		12·80	13·87	15·56

Ethyl alcohol was found to be practically ineffective; even at the high concentration of 1 part in 6 reduction in growth was negligible. Butyl alcohol was toxic at this concentration, but allowed of quite good growth when it was present at the concentration of 1 part in 21 R.S. Now the quantity of alcohols demonstrable in the distillate from stale medium is very much smaller than this. A solution of alcohol of 1 part in 41 smells very strongly alcoholic and gives a copious precipitate in the iodoform test, while the iodoform precipitate in the distillate was very small, though the smell was unmistakable. Acetaldehyde, too, does not inhibit growth unless it is present in comparatively high concentrations, considerably more than the doubtful trace thereof found in the distillates.

Thus alcohols or aldehydes alone are not very potent stalers and can play no measurable part in the staling of *Fusarium* cultures.

In the case of organic acids it must be borne in mind that the results obtained have to be compared with growth in the acidified stale medium. This is approximately 4, when the value for the control is 15 (cf. Table VI, where growth in the acidified medium is 2·35 when the control is 9·16, which is equivalent to a growth of 3·87 when the control is 15).

In the preliminary experiments varying amounts of normal acetic acid and propionic acid solutions were added to 100 c.c. of Richards' solution (see Table XIV).

TABLE XIV.

Preliminary Experiments. Effect on Growth of Botrytis cinerea of Addition of Fatty Acids.

R.S. + N acid solution.	*Acetic acid + R.S. Bot. growth.*	*pH acetic + R.S.*	*Propionic acid + R.S. Bot. growth.*	*pH Propionic + R.S.*
100 c.c. + 2 c.c.	0 +	3·8	0	3·8
100 c.c. + 1·33 c.c.	1·83	3·9	0 +	3·9
100 c.c. + 1·143 c.c.	0·71	4·0	0 +	4·0
100 c.c. + 1 c.c.	1·32	4·0	0·845	4·0
Control R.S.	10·15	4·6	10·15	4·6
100 c.c. + 1 c c.	2·13	3·8	0 +	4·2
100 c.c. + 0·5 c.c.	3·76	4·0	3·9	4·4
100 c.c. + 0·25 c.c.	8·36	4·1	6·23	4·4
100 c.c. + 0·125 c.c.	8·78	4·2	8·49	4·6
Control R.S.	9·0	4·6	9·28	4·6

It will be seen that relatively low concentrations of acetic and propionic acids greatly reduce the mean germ-tube length. At about 0·03 per cent. acetic acid (0·5 c.c. N acetic acid to 100 c.c. R.S.) growth is considerably less than the control, and at 0·063 per cent. growth is about equal to that in an acidified stale medium. When the concentration of acetic acid is again doubled growth is practically stopped. These results can in no case be attributed to increased acidity, since the pH value lies between 3·8 and 4·6. Propionic acid has the same effect, but appears to act at somewhat lower concentrations.

These results suggested that a study of the growth-retarding powers of a larger number of organic acids might be of value. In testing the effect of various concentrations of organic acids, the acids were added, not to the full-strength medium, but to R.S./100, as in that way the conditions in the acidified stale medium were more closely approximated to, experiments having shown that the sugar content of the staled liquid was in the neighbourhood of R.S./100.

The primary object of the experiments was to compare the effects of different acid radicals; hence the acidity might be neglected, since it was very nearly the same in every case. The acidity ranged from pH = 4·6 to pH = 3·3, and thus fell in the region where germination (see Webb) and growth of *Botrytis cinerea* are not greatly affected. The spores of this fungus are known to be tolerant to hydrogen ions over a comparatively wide range of concentration from pH = 3·1 to neutrality. The differences of germ-tube length, due to pH variation itself, observed within the range of pH of the experiments described here, fall within the experimental error.

The following acids were used:

Fatty acids: Acetic, propionic, butyric, valeric, glycollic (hydroxyacetic), and lactic (hydroxypropionic).

Dicarboxylic acids: Oxalic, malonic, succinic, and glutaric.

Aromatic acids: Benzoic, salicylic (1·2 oxybenzoic), dioxybenzoic (1 : 2 : 5), and gallic (1 : 3 : 4 : 5 trioxybenzoic).

Approximately fifth-normal solutions of the fatty and the dicarboxylic acids were made up and standardized. Varying amounts of these acid solutions were added to 10 c.c. R.S./100, namely 2·0 c.c., 1·0 c.c., 0·5 c.c., 0·25 c.c., 0·125 c.c., giving approximately N/27·5, N/55, N/105, N/205, N/405 strengths of acids respectively in the nutrient solutions. Drop-cultures with spores of *Botrytis cinerea* were set up, and the germ-tubes measured in the usual way.

Since the aromatic acids are much less soluble, it was not found practicable to make up N/5 solutions, except of gallic acid. Therefore the appropriate amount of each acid was weighed out and dissolved in 100 c.c. R.S./100, so as to give a N/100 solution in R.S. Dilutions were obtained by adding 50 c.c. R.S./100 to 50 c.c. of the N/100 solutions, giving N/200, and by adding 50 c.c. R.S./100 to 50 c.c. of this N/200, giving N/400, and so on.

The solutions of benzoic, salicylic, and dioxybenzoic acid were found to be somewhat more acid than those containing fatty acids, therefore corresponding series were carried out in each case in which N/10 sodium hydroxide was added, drop by drop, until the pH = 4·6 (see Table XVII).

In the tables given (XV, XVI, and XVII) the actual experimental values of the mean germ-tube lengths are all reduced so as to correspond with a control value of 15. This is necessary here for the purpose of comparison of the effects of different acids. In other parts of this paper the experimental values are given.

TABLE XV.

The Effect on Botrytis Growth of Addition to Richards' Solution of Organic Acids.

Strength of acid in R.S.	*N*/27·5	*N*/55	*N*/105	*N*/205	*N*/405
Acetic		2·08	5·69	10·32	11·29
,,	0	1·65	5·93	8·88	10·2
,,		0+	3·5	7·35	9·35
Propionic		0+	0+	4·22	8·04
,,	0	0·5	1·61	4·1	9·27
Butyric	0	0	0·7	2·37	5·71
,,	0	0·7	2·54	4·02	6·2
Valeric		3·76	5·38	6·66	12·17
,,	0	4·7	6·75	10·03	12·38
Glycollic		10·72	11·53	12·46	15·7
Lactic		8·0	9·28	10·38	11·3
Malonic	5·9	7·26	10·37	11·3	
,,	6·71	9·17	10·27	12·69	
,,	4·18	8·08	10·45	10·09	
Succinic	10·16	11·02	13·38	14·15	
,,	8·72	10·78	11·29	—	
Glutaric	9·88	12·38	—	15·09	
Strength of oxalic acid in R.S.	*N*/19·3	*N*/38·6	*N*/73·7	*N*/143·9	*N*/284·3
Oxalic	0	1·44	—	7·5	9·37
,,		2·73	5·2	10·42	10·58
,,	0·54	2·79	5·06	8·52	10·2

TABLE XVI.

The Effect on Botrytis Growth of Aromatic Acids.

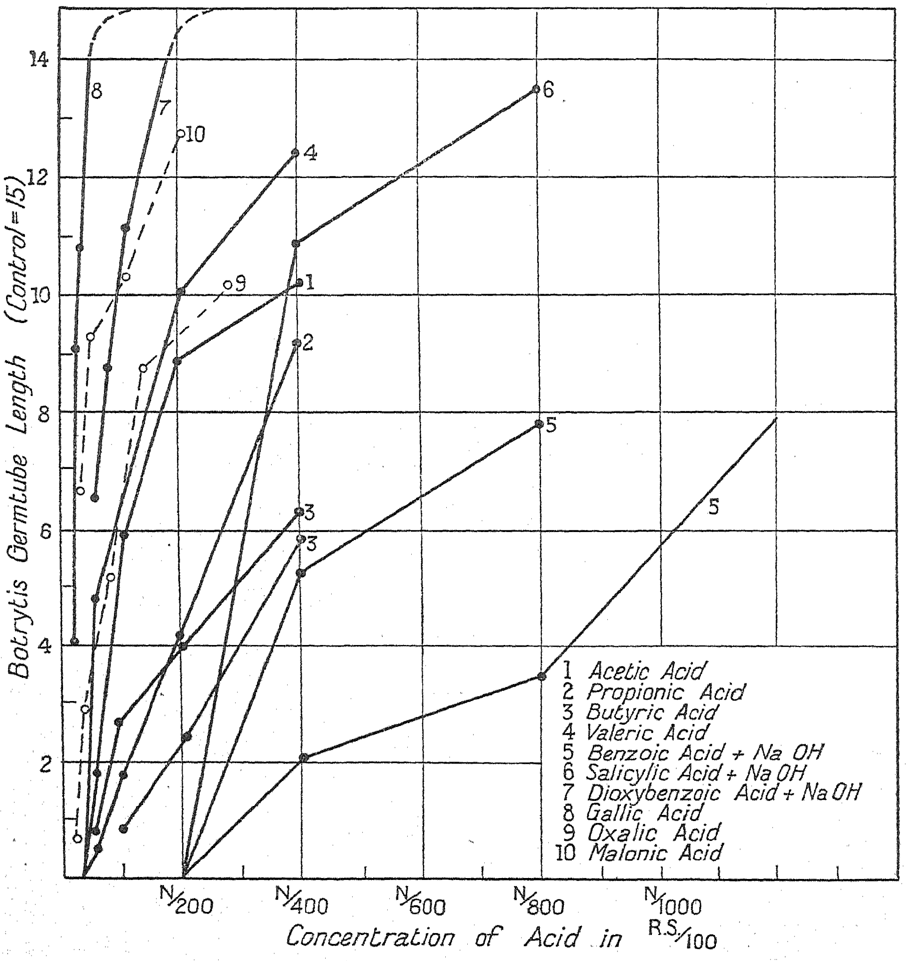
Strength of acid in R.S.	*N*/100	*N*/200	*N*/400	*N*/800	*N*/1000	*N*/1600	*N*/2000	*N*/3200	*N*/4000	*N*/6400	*N*/8000
Benzoic	0	0	0	0		7·80		8·34		12·05	
Salicylic	0	0	0	0	0		8·32		12·65		as control.
Dioxybenzoic	0	1·56	5·97								
,,	0	1·24	4·6	8·41		11·49		13·63			,,
Gallic	as control.	as control.	as control.								

These reduced values are used in the graph of the results in which the ordinates represent acid concentrations in Richards' solution N/100, N/200, N/300, &c., and the abscissae represent *Botrytis* germ-tube lengths in divisions of the micrometer eye-piece (see figure on p. 590).

TABLE XVII.

The Effect on Botrytis Growth of Aromatic Acids when pH = 4·6.

	$\frac{N}{55}$	$\frac{N}{72}$	$\frac{N}{100}$	$\frac{N}{200}$	$\frac{N}{400}$	$\frac{N}{800}$	$\frac{N}{1600}$
Benzoic			0	0	5·17	7·73	
,,			0	0	2·08	3·45	12·28
Salicylic			0	0	10·92	13·6	
Dioxybenzoic	6·56	8·81	11·21	as control.	as control.		



The effect of various organic acids on *Botrytis* growth.

It is at once evident that aromatic acids, benzoic and salicylic, are toxic even after the addition of sodium hydroxide, at much lower concentrations than are any of the fatty acids. Growth is entirely inhibited by either at N/200, and reduced to 4 (which is taken as representing growth in the acidified stale medium, see Table VII) at about N/500 in the case of benzoic acid + NaOH, and at about N/275 in the case of salicylic acid + NaOH. Dioxybenzoic acid + NaOH is much less toxic than the acid alone, this being due, probably, to the marked acidity of the latter (pH = 2·2–3·4). Gallic acid is but slightly toxic at high concentrations (N/10).

Among the fatty acids distinct differences in toxicity are shown, propionic and butyric acids being effective at lower concentrations than acetic acid, while the next higher member of the series, valeric acid, is less toxic than acetic acid. The introduction of a hydroxyl into the acid radical seems to reduce very largely its toxicity; N/55 glycollic (hydroxyacetic) and lactic (hydroxypropionic) acids have but little effect.

It is interesting to note that oxalic acid, which has been generally supposed to be a very deleterious plant product, is somewhat less effective in reducing *Botrytis* growth than is acetic acid. The toxicity of the dicarboxylic acids rapidly decreases when passing up the series, malonic, succinic, glutaric, with the introduction of hydroxyl into the radical. A similar decrease in toxicity, with increase in hydroxyl, is shown by glycollic (hydroxyacetic) and lactic (hydroxypropionic) acids, and by the aromatic acids, salicylic, dioxybenzoic, gallic.

From the graph it is possible to find the approximate concentrations of acids in Richards' solution, which are required to reduce the growth to a value equivalent to that in the acidified stale medium (= 4):

Benzoic + NaOH . . .	N/500	Oxalic	N/55
Salicylic + NaOH . . .	N/275	Valeric	N/50
Propionic	N/200	Malonic	N/25
Butyric	N/200	Dioxybenzoic + NaOH	N/25
Acetic	N/75	Gallic	N/10

Similarly, the approximate concentrations required for inhibition of growth are found to be:

Benzoic + NaOH .	N/200
Salicylic + NaOH .	
Butyric	N/50
Acetic . . .	N/30
Propionic	
Valeric	
Oxalic	N/20

Now, it has been found, by testing different dilutions, that it is practically impossible to distinguish between these fatty acids in dilute solution, and, indeed, impossible to demonstrate their presence by means of ferric chloride. However, the ester test (sulphuric acid and alcohol) will show the presence of organic acids in fairly dilute solution, for example, in N/100 propionic acid. The presence of organic acids in the stale medium may be demonstrated by the ester test, when the fruity smell is much stronger than with

N/100 propionic acid. Thus it may be assumed that organic acid radicals are present in greater concentration than that corresponding to N/100, quite probably as much as N/50. As has already been shown, a large number of the organic acids examined cause considerable reduction in *Botrytis* growth at lower concentrations than this.

The general conclusion from these experiments is that organic acids of the fatty acid series are sufficiently toxic to explain the fact that, even after it has been acidified, a used medium is still 'stale', i. e. permits only reduced growth of *Botrytis* spores. Moreover, these acids are produced by *Fusarium* in sufficient concentration to give this effect.

Aromatic acids, which, however, have not been demonstrated in the stale medium, are more toxic than fatty acids, while oxalic acid is somewhat less deleterious than acetic acid.

The introduction of hydroxyl groups into the acid radical, both fatty and aromatic, progressively decreases their toxicity; indeed they then become available as sources of food.

The conclusions of Waterman (1915) afford interesting parallels with these. His aim was to determine the nutritive value of aromatic and fatty acids as the sole source of carbon for *Penicillium glaucum.* This fungus can grow on *p*-oxybenzoic, *m*-oxybenzoic, protocatechuic (1:2:4 dioxybenzoic), and gallic acid. Salicylic acid (*o*-oxybenzoic) has a very slight nutritive value, while 1:2:5 dioxybenzoic (that used in the present experiments) is not assimilated. Acetic acid is, he found, the best nutrient of the fatty acids; the highest fatty acids have an injurious action. Succinic acid is a good source of carbon; oxalic and malonic have a poor value. He states that the introduction into the molecule of a methyl group, in general, reduces the nutritive value of a compound, and that the injurious action of a compound is generally reduced by introduction of the— OH or COOH group. This last conclusion finds an exact parallel in the work described in this paper.

Waterman explains his results on the basis of the difference in the oil/water partition ratio of the acids; a high oil/water ratio, with appreciable solubility in water, corresponds with a high injurious action.

SUMMARY.

1. The literature on the subject of the staling of fungi in culture is surveyed.

2. *Fusarium* sp. was grown on Richards' solution, and the 'staleness' examined by the *Botrytis* germ-tube method.

3. Exhaustion of food is not of primary importance, as is shown by *Botrytis* growth in dilutions of the stale medium, and in dilutions of the fresh medium, and by quantitative determination of the remaining sugars.

4. Boiling brings about partial restoration of a stale medium when the culture is still acid. Acidifying will effect partial improvement in older alkaline cultures. The result of acidifying and boiling the same liquid is greater than the sum of these two separately. Staling is not considered to be due to an enzyme which is here deactivated by heating.

5. Extraction with ether after acidifying, but not before, removes the staleness. This is evidence against the presence in the alkaline medium of free organic nitrogen bases, and for that of the salts of organic acids.

6. Oxidation of the medium with hydrogen peroxide and with gaseous oxygen was ineffective in removing staleness.

7. Charcoal is capable of considerable absorption of the toxic substances, but this is not made manifest till alkalinity is removed. Treatment with colloidal clay ('Collosan') gives growth very nearly as good as in the control. It makes no difference whether the liquid is acidified before or after treatment.

8. Chemical tests indicate the production by *Fusarium* growing on Richards' solution of ammonia, small amounts of alcohol, and of salts of fatty acids, acetic, propionic, butyric, valeric, and lactic, and, possibly, of traces of aldehydes and formaldehyde.

9. The presence in the stale culture of large crystals of insoluble magnesium ammonium phosphate shows that fairly large amounts of ammonia are produced, but that it is nearly all deactivated. The amount of free ammonia is small.

After the alkaline stale medium has been boiled, it is found to be more strongly alkaline. This final alkalinity is not due to ammonium compounds.

10. The growth of *Botrytis* spores is affected by the addition to the fresh medium of various organic compounds which have been demonstrated in the stale medium. Alcohols and acetaldehyde are toxic only at high concentrations; they can play a very small part, if any, in 'staling'. Simple organic acids are toxic at sufficiently low concentrations to be responsible for the poor germinative capacity of the acidified stale medium. A graph is given, in which the toxic effects of a number of organic acids are compared.

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NOTE.

PHOTOTAXIS AND THE PRESSURE OF LIGHT.—The idea of phototaxis as a biological property of the lower organisms was established by Strasburger in the year 1878, and since then this phenomenon has been studied by many investigators. However, no one has attempted to define the nature or to discover the cause of this remarkable phenomenon. All investigators of phototaxis recognize it as a manifestation of the peculiar power of protoplasm to react to the stimulus of light; the organism, under the influence of this stimulus, actively moves towards or away from the light. I think that every naturalist will agree with the thesis that at the bottom of any physiological property of whatever organism there must lie some kind of physico-chemical cause. Such a cause we must seek in the present instance, with the object of explaining the appearance of phototaxis.

The cause of phototaxis must lie in the interaction of the light-energy and the construction of the body of the organism, as well the apparatus (of course, living), which reveals the action of the light-force on it, always in accordance with its organization, i. e. with the construction of the plasm and like organs or organoids of the cell.

The nature of light-energy is still far from being completely solved, and still more remote is the solution of the problem of the nature of the living protoplasm.

Physicists have investigated the question of the mechanical pressure exerted by light-rays. This question is an old one, and was first raised by Kepler in the year 1608, with the object of explaining the formation of the tails of comets.

Theoretical considerations of the pressure of light were expressed many years ago by Maxwell, and in more modern times the idea of light-pressure has received a real confirmation in the experiments of Professor Lebedeff.[1]

Schwarzschild,[2] by a mathematical analysis, has shown that with black bodies of extremely small dimensions, the diameter of which is comparable with the wave-length of light, the pressure of light is equal to the force of gravity; with a still further decrease in diameter, the light-pressure may be eighteen times that of gravity. Schwarzschild states: 'Im Normalfall wird der Druck des Lichtes gleich der Schwerkraft, sobald der Kugeldurchmesser bis auf $2{,}5\lambda = 1{,}5\mu$ herabsinkt. Bei weiterer Verkleinerung der Kugel wächst der Druck über die Schwerkraft hinaus, bis er sie bei einem Kugeldurchmesser von $0{,}3\lambda = 0{,}18\mu$ um das 18-fache übertrifft. Von diesem Maximalwert sinkt er schnell wieder ab und wird bereits für den Kugeldurchmesser $0{,}12\lambda = 0{,}07\mu$ wieder der Schwerkraft gleich, um sich sodann rasch der Null zu nähern.'

The question arises as to whether the key to the solution of the mechanism of phototaxis is to be found in this property of light.

The idea of the pressure of light-rays, as a mechanical cause of phototaxis, seems a possible one. If we suppose that the construction of the phototactic *Chlamydomonas* and of green zoospores is of such a nature that the fate of the rays of light falling on to it varies in different parts of the body—in some

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[1] Lebedeff: Ann. d. Physik, 4. Folge, Band 6, S. 433.

[2] Schwarzschild: Der Druck des Lichtes auf kleine Kugeln und die Arrheniussche Theorie der Cometenschweife. Sitzungsber. d. mathem.-physical. Klasse der Königl. Akademie der Wissenschaften zu München, 1901, S. 335.

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parts the light is reflected, while in others it is entirely absorbed by the minute elements of the chlorophyll bodies, not exceeding 1 μ in diameter, and lastly, some of the rays pass right through the body, without being either absorbed or reflected, and so on—one may imagine that, under the influence of the uneven diffusion of the light-rays on the surface and inside the organisms, the pressure of the light on the various parts of the body will also be different. As in the case of so-called geotaxis, where the direction of motion depends on the position of the centre of gravity, so in the case of phototaxis it is possible *a priori* to suppose a special centre of the pressure of light. In order to explain positive phototaxis, it is indispensable to assume a centre of application of the force of light situated at the posterior end of the body of the organism, for in this case, in whatever way the actively moving organism turns, the position of this centre will always bring the organism with the front part of its body towards the source of light. On such an assumption it is easy to explain the difference in the sensitiveness to light of different organisms, since the sensitiveness is in this way made dependent on the position of this centre. Observation of organisms showing phototaxis at the time when they are moving in an almost straight line towards a concentrated source of light (as in the case of observations taken in a hanging drop illuminated by the sun-rays) always reveals a wavering of the body of the organism, i.e. the organism does not move in a perfectly straight line, but in a zigzag manner, as if every effort of it to the side were met by a resisting force which immediately forced it back again. In this way positive as well as negative phototaxis can be explained. Both the degree of geotactic sensibility and the degree of phototactic sensibility can in this way be made directly dependent on the position of the centre of the application of the force, which is with geotaxis the centre of gravity, and with phototaxis the centre of application of the force of the light-rays.

The *concentrated light* which is obtained in the focus of a drop of water is absorbed by the organism, but does not kill it, as it cannot heat the microscopic organism contained in the hanging drop in consequence of the comparatively great surface of body compared to mass. The organism under these conditions does not throw off its cilia, as occurs in the case of the heating of the whole drop.

The question why some organisms do not exhibit phototaxis (e.g. *Parmaecium* and other infusoria in the experiments of Sosnowsky, Jensen, and others) is not difficult to explain if we bear in mind that the pressure of light is proportional to the light-energy absorbed by the body. The infusoria are transparent to a considerable degree, and the organization of their bodies may be of such a kind that inside the bodies there is not, and cannot be, formed a centre of application of the force of light, and therefore they are not subject to the mechanical action of light.

This suggestion of the relation between phototaxis and the pressure of light appeared in my book (published in Russian in Warsaw in 1904) entitled *Materials for the Study of the Morphology and Physiology of Green Algae*, all the remaining copies of which were lost in Warsaw in 1915.

V. F. CHMIELEVSKY.

Botanical Institute, University,
Rostov-on-Don, Russia.

The Staling of Fungal Cultures.

II. The Alkaline Metabolic Products and their Effect on the Growth of Fungal Spores.

BY

CLARA A. PRATT, M.A., PH.D.

(*Department of Plant Physiology and Pathology, Imperial College of Science and Technology London.*)

With one Figure in the Text.

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INTRODUCTION.

IT has already been demonstrated in a previous paper (1) that the organic acids which *Fusarium* produces will account for the fact that an acidified stale medium permits only reduced growth of *Botrytis* spores. The question then arises, as to whether these acids represent part of the 'staling principle', or whether a growth-inhibiting factor, which does not exist in the alkaline medium, has been introduced by acidification. It is conceivable that the toxicity of organic acids may be due to the un-ionized part of the slightly dissociated free acids, and that the ions of the highly dissociated potassium salts may have little or no toxicity. This was tested

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by an experiment (Table I), in which propionic acid was added to diluted Richards' solution (R.S./100) so that the acid concentration was N/105, and a range of hydrogen-ion concentrations was obtained by adding drops of a potassium hydroxide solution. As a control a similar range was obtained by adding KOH to R.S./100.

TABLE I.

pH varied by Addition of Potash Solution.

pH	3·2	4·6	6·0	7·0	8·0	9·0
R.S./100 *Botrytis* growth	11·10	10·64	9·76	10·17	6·97	7·78
R.S./100 + HPrN/106 *Botrytis* growth.	0	1·13	7·45	11·30	8·62	6·93

The results show that on the alkaline side of neutrality the organic acid is ineffective in reducing growth. Moreover alkalinity due to potash, i. e. hydroxyl ions, is but slightly effective, so that mere pH value does not appear to be a growth factor within very wide limits.

Thus the alkaline medium must be studied in order to discover the essential staling principle.

ORIGIN OF THE ALKALINITY.

The experiments described below aimed at throwing some light on the origin (or cause) of the alkalinity developed by cultures of *Fusarium* on Richards' solution.

i. Omission of Different Constituents of Richards' Solution.

A series of flask cultures was set up, in each of which one of the constituents of normal Richards' solution was omitted. At regular intervals the contents of a flask in each series were filtered, the dry weight of the mycelium determined, and the pH of the filtered medium found. The results are set out in Table II, together with those of a control series with normal Richards' solution.

TABLE II.

	Days stale.	*pH.*	*Dry Wt.* grm.		*Days stale.*	*pH.*	*Dry Wt.* grm.
A.	0	4·6	0	B.	0	4·2	0
	7	4·6	0·0506		8	4·0	0·0026
	10	5·2	0·1866		14	3·8	0·0194
	14	6·2	0·1212		15	4·1	0·0107
	15	6·6	0·3377		17	4·1	0·0274
	17	7·0	0·4193		22	3·8	0·0737
	22	7·4	0·5425		30	4·0	0·0771
	30	8·6	0·6070				
	36	9·0	0·6102				

TABLE II (*continued*).

	Days stale.	*pH.*	*Dry Wt.* grm.		*Days stale.*	*pH.*	*Dry Wt.* grm.
C.	0	6·4	0	D.	0	4·6	0
	7	6·6	0·0170		8	6·8	0·0301
	9	7·2	0·0171		12	7·2	0·0660
	13	8·2	0·0379		13	7·0	0·0520
	14	8·1	0·0287		15	7·2	0·0405
	16	8·1	0·0280		19	7·4	0·0655
	21	7·8	0·1098		21	7·6	0·0720
	31	7·8	0·0908		26	7·6	0·0605
					29	8·4	0·0530

E. No measurable growth.

Medium in A. R.S.
,, B. R.S. without KNO_4
,, C. R.S. ,, KH_2PO_4
,, D. R.S. ,, $MgSO_3$
,, E. R.S. ,, cane sugar.

There was no development of alkalinity in B, from which the potassium nitrate was absent; naturally the growth was small, but no smaller than when the phosphate or the sulphate was absent, in both of which cases the culture became alkaline. It is noteworthy, too, that in C and D alkalinity is developed much more rapidly than in the control (A) and with an amount of mycelium which produces no alteration of pH in the control.

No measurable growth was obtained in the absence of sugar.

The conclusion is drawn that the nitrogenous material, at any rate in this particular medium, is responsible for the development of alkalinity.

ii. Dilution of the Nitrogen Source.

Similar flask cultures were set up containing Richards' solution in which the potassium nitrate alone was reduced in amount.

TABLE III.

	Days stale.	*pH.*	*Dry Wt.* grm.		*Days stale.*	*pH.*	*Dry Wt.* grm.
A.	0	4·2	0	B.	0	3·8	0
	7	4·6	0·0910		7	4·2	0·0673
					10	5·0	0·1460
	13	6·2	0·3037		13	5·8	0·1944
					17	5·8	0·2133
	21	6·6	0·2855		21	5·8	0·1633
	24	7·0	0·3002		24	6·2	0·2416
	28	7·0	—		28	6·3	—
	44	8·8	—		44	8·6	—

TABLE III (*continued*).

	Days stale.	*pH.*	*Dry Wt.*		*Days stale.*	*pH.*	*Dry Wt.*
			grm.				grm.
C.	0	3·8	0	D.	0	4·0	0
	7	4·0	0·0575		7	3·7	0·0401
	10	4·2	0·0970		10	3·8	0·0612
	13	4·4	0·1096		13	4·0	0·0842
	17	5·2	0·1812		17	4·0	0·1030
	21	5·4	0·1402		21	4·0	0·0950
	24	6·4	0·2790		24	5·4	0·1572
	28	5·2	0·1172		28	6·0	0·2235
	44	6·0	—		44	5·6	0·1398

A. R.S. with 10 grm. KNO_3 per litre (Normal strength).
B. ,, 5 ,, ,, ,, ,,
C. ,, 2·5 ,, ,, ,, ,,
D. ,, 1·25 ,, ,, ,, ,,

Cultures were taken at intervals and the value of the pH and the dry weights of mycelium determined.

The results (Table II) show that the pH value increases more slowly as the amount of potassium nitrate supplied is reduced. Moreover, reduction in concentration of nitrogen source produces a smaller growth of fungus, so that change in reaction can be correlated with amount of growth. There is, then, a close relation between the amount of available nitrogen (i. e. potassium nitrate), the amount of mycelium produced, and the change of hydrogen-ion concentration.

It should be noted that the development of alkalinity is not a feature of all cases of staling. For example, in a set of cultures where the medium was Richards' solution with a quarter of the normal concentration of potassium nitrate, the pH value did not become greater than 6·2 in six weeks, although the medium was completely staled in four weeks. In another case where the potassium nitrate had been replaced by 0·264 per cent. asparagin, i. e. about one-third the usual concentration of nitrogen, there was no change of pH, nevertheless the medium, twenty-eight days' old, inhibited the growth of *Botrytis* spores.

When, however, potassium nitrate was replaced by equivalent amounts of asparagin, ammonium nitrate, or calcium nitrate, the cultures became alkaline in three or four weeks.

iii. *Estimations of Carbonates.*

A number of estimations of carbonates in stale Richards' solution were carried out. The medium was treated with dilute sulphuric acid and the carbon dioxide given off absorbed by means of soda-lime and weighed directly (Fresenius-Claussen method) or passed into a standard baryta solution (Pettenkofer method). This gave the total carbon dioxide in the medium. The apparatus used in both methods was tested with N/10 sodium carbonate solutions. Carbonates alone were estimated by first boiling a known volume of the medium to drive off any dissolved carbon dioxide and then determining the amount of combined carbon dioxide. Dissolved carbon dioxide was estimated by the Pettenkofer method by passing a current of CO_2 free air through the medium (to which no acid was added). In some cases, acid was then added to the samples of medium thus freed from dissolved carbon dioxide and the combined carbon dioxide determined, with the unexpected result that these values were higher than those obtained when the medium was boiled to drive off carbon dioxide.

TABLE IV.

Results by Fresenius-Claussen Method.

Carbon Dioxide in 100 c.c. Stale Medium.

Days stale.	*Total CO_2 grm.*	*Days stale.*	*Combined CO_2 grm. (by Boiling).*
116	0·0864	117	0·0304
116	0·0768	117	0·0256
118	0·0904	117	0·0288
118	0·0936	123	0·0384
		123	0·0308
		113	0·0512
		132	0·0340

TABLE V.

Results by Pettenkofer Method.

Carbon Dioxide in 100 c.c. Stale Medium.

Days stale.	*Total CO_2.*	*Dissolved CO_2.*	*Combined CO_2.*	*Combined (by Boiling first).*
	grm.	grm.	grm.	grm.
170	0·0525			
171		0·01125		0·06
175		0·0025		0·04125
177	0·06166			
56	0·1083			0·040
57				0·04833
60	0·07625			0·04875
61				0·0385
62	[0·0560]	0·0135	0·0425	0·03875
63	[0·10125]	0·0125	0·08875	
67	[0·08625]	0·01125	0·075	0·05
69	[0·08775]	0·01125	0·0765	
70	0·0966	0·00375		

(Figures in square brackets [—] obtained by addition.)

Now, the fact that the boiled liquid was found to contain only about one-half of the amount of combined CO_2 present in the unboiled liquid suggests that a bicarbonate (which is converted into carbonate on boiling with loss of half its CO_2) is chiefly formed. On general consideration, too, a bicarbonate—presumably potassium bicarbonate in the case of Richards' solution—is likely to be present, rather than the carbonate, since there must be in the medium an excess of CO_2 from respiration.

Examination of the figures of Tables IV and V gives the following results:

	per cent.
(i) Mean total CO_2	0·083
(ii) Mean dissolved CO_2	0·009
(iii) Difference between (i) and (ii) combined CO_2	3·073
(iv) Mean combined CO_2 after boiling (exptl.)	0·040
(v) Difference between (iii) and (iv)	0·033
(vi) =(v) multiplied by 2, i. e. CO_2 as bicarbonate in stale medium	0·0655
(vii) Difference between (iv) and (v), i. e. CO_2 as carbonate in stale medium	0·008

As will be shown below, the amount of bicarbonate found in the stale medium is more than sufficient to account for the alkalinity developed.

THE EFFECT ON THE GROWTH OF FUNGAL SPORES OF THE ALKALINE PRODUCTS OF METABOLISM.

The alkaline metabolic products of *Fusarium* on Richards' solution are, then, potassium carbonate and bicarbonate, the latter being formed in much greater amount; also free ammonia, of which small amounts have been demonstrated (3).

Attempts were made to reproduce the alkalinity of the stale medium by adding to R.S./100 various amounts of ammonia, carbon dioxide, and potassium bicarbonate solutions, and the growth of *Botrytis* in these liquids was compared with growth in R.S./100 made alkaline with potash. Experimental details and results are tabulated below:

TABLE VI.

Treatment of Medium.	*Botrytis Growth.*
(1) KOH till pH = 8·4	10·58
(2) 12 c.c. N/10 NH_4OH per 100 c.c. R.S./100, (pH then 10+)	
CO_2 passed till pH = 8·4	11·85
(3) $KHCO_3$ till pH = 8·4	0·24
(4) 8 c.c. N/10 NH_4OH per 100 c.c. R.S./100 (pH then 10+)	
CO_2 passed till pH = 7·0	
$KHCO_3$ till pH = 8·4	0·27
(5) KOH till pH = 10+	
saturated with CO_2 (pH then 6·0)	
KOH till pH = 8·4	2·03

It will be seen (2) that ammonia and CO_2 have no effect on *Botrytis* growth, but the addition of potassium bicarbonate to the medium gives very reduced growth (3) compared with a solution of the same pH produced by potash (1). The addition of ammonia when potassium bicarbonate is present, as in (4), has no further effect.

In (5) the formation of potassium bicarbonate by passing CO_2 into the liquid made alkaline with potash was attempted. Although it was uncertain how much bicarbonate or how much carbonate had been produced, yet growth was much reduced.

Ammonia does not seem to be an active staling substance in the case of Richards' solution. Potassium bicarbonate is a very potent growth inhibitor; this is quite independent of its pH value, for growth is not sensibly reduced when the same pH is obtained with potash.

In order to find out the amount of bicarbonate required to produce this effect, a molecular solution of potassium bicarbonate was added to 100 c.c. quantities of R.S./100 and a range of hydrogen-ion concentrations obtained. The *Botrytis* growth in these solutions was compared with that in R.S./100 made alkaline with potassium carbonate and with potash.

TABLE VII.

Molecular Solution added.	*Amount added to 100 c.c. R.S./100.*	*Botrytis Growth.*	*pH before Boiling.*	*pH after Boiling.*
$KHCO_3$	0·1 c.c.	8·21	6·6	7·8
,,	0·2 c.c.	7·01	7·0	9·2
,,	0·45 c.c.	3·28	7·8	10
,,	1·35 c.c.	0·38	8·4	10
K_2CO_3	½ drop	9·68	6·6	7·0
,,	1 drop	10·08	7·0	7·0
,,	1½ drops	8·24	8·8	8·8
KOH		9·32	6·6	6·6
,,		9·55	7·0	7·0
,,		9·5	7·8	7·6
,,		10·24	8·4	8·0

As will be seen in Table VII, potassium carbonate has no more effect than potash (i.e. pH value alone), so that any carbonate present in a stale medium need not be considered as a growth inhibitor.

As it seemed possible that some of the ammonia might be driven out of the solution by the mechanical action of rapidly passing carbon dioxide, as used above (Table VI), and, moreover, since the carbon dioxide shifted the pH so rapidly in the acid direction that it was difficult to stop the process at the required point (pH 8·0–8·4), the following method was adopted:

Varying quantities of a standard (N/10) solution of ammonia were added to 100 c.c. portions of R.S./100; of each 100 c.c., two parts were put into cylindrical vessels, 25 c.c. capacity (actually weighing bottles). Several drops of an indicator, cresol red, were added to the liquid in one of the bottles and both were placed in a large vessel, of the kind used as a desiccator, in an atmosphere of carbon dioxide. A slow stream of carbon dioxide from the generator was passed through the desiccator. Gradually the liquids absorbed the carbon dioxide, so that in about half an hour, the time varying with the concentration of ammonia, the indicator began to change colour. When the indicator colour showed that the pH was about 8·4, the corresponding bottle, without indicator, was removed from the desiccator, the pH of part of the liquid determined, and the rest used for a *Botrytis* growth test.

Several ammonia estimations of the liquids, before and after the treatment with carbon dioxide, were carried out, and it was found that the procedure did not cause any appreciable loss of ammonia.

Drops of the medium containing ammonia, set up in the usual way, showed good growth, even when the amount of ammonia added was considerably greater than that estimated in the stale medium (3). However, the value of these observations is uncertain since a large proportion of the ammonia probably diffused into the container; the drops were less alkaline (pH about 8·0) at the end of the experiment than at the beginning (pH = 10+). Still, this loss of ammonia would take place from drops of the stale medium under similar conditions.

Table VIII.

Effect on Botrytis Growth of Ammonium Bicarbonate.

*Amount N/*10 *Ammonia Solution added to* 100 *c.c. R.S./*100.	*pH.*	*Botrytis Growth.*
1 c.c.	7·6	Long
2 c.c.	7·6	Long
4 c.c.	8·0	Long
6 c.c.	8·2	Long
8 c.c.	8·2	Long
12 c.c.	8·0	9·43
24 c.c.	8·2	7·97
4·8 c.c. (N)	8·4	5·74
9·6 c.c. (N)	8·4	0

('Long' = 20 scale divisions and over; the germ-tubes generally branched.)

Ammonia neutralized by carbon dioxide, presumably chiefly ammonium bicarbonate,[1] retards *Botrytis* growth, as shown in Table VIII, though not to an extent comparable with potassium bicarbonate; for example, at a certain concentration (12 c.c. N/10 per 100 c.c. of medium) growth is about half that in the control, while an equivalent concentration of potassium bicarbonate practically inhibits growth (see Table VII). A comparatively high concentration of ammonium bicarbonate (9·6 c.c. N solution per 100 c.c.) is required to inhibit growth entirely.

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[1] Chemical evidence was obtained of the presence of bicarbonate in the liquid.

This is probably due to the fact that ammonia is a much weaker base than potash, so that ammonium bicarbonate in solution is less dissociated than potassium bicarbonate, consequently a lower concentration of bicarbonate ions (HCO_3) would be derived from a solution of the ammonium salt than from one of equivalent strength of the potassium salt.

The presence of potassium bicarbonate would explain a striking characteristic of the stale medium, namely that, on boiling, the alkalinity of the liquid is much increased (3). In the case of the bicarbonate alone, as shown in the last column of Table VII, boiling brings about increase in alkalinity comparable with that in the stale medium. The slightly alkaline bicarbonate is converted into the strongly alkaline carbonate.

In order to determine with greater accuracy how much potassium bicarbonate was required to bring 100 c.c. of R.S./100 to pH = 8·2, a number of titrations with M/10 potassium bicarbonate solution were carried out using various indicators. Brom thymol blue gave the neutral point (pH = 7·0) and, by subtraction of the results obtained with cresol red and thymol blue (pH = 8·2), the free bicarbonate required to produce an alkalinity of pH = 8·2 was obtained.

TABLE IX.

Indicator.	*pH at End Point.*	*M/10 $KHCO_3$ c.c. per 100 c.c. R.S./100.*
(1) From thymol blue	7·0	2·16
(2) Cresol red	8·2	13·02
(3) Cresol red	8·2	13·04
(4) Thymol blue	8·0–8·2	12·84
(5) Means of (2) and (4)	8·2	12·96
(6) Difference between (5) and (1)		11·1

Thus approximately 1·3 c.c. of a molecular solution of potassium bicarbonate must be added to 100 c.c. R.S./100 to obtain a pH of 8·2. This result was used in the experiments described in Table X, in which increasing amounts of potassium bicarbonate were added to R.S./100. The effects on growth of sodium bicarbonate and of ammonium bicarbonate, since this compound might be present in stale liquids, were also studied.

Doubling the amount of potassium bicarbonate (i. e. raising to 2·6 c.c.) was found to inhibit growth entirely. Sodium bicarbonate is nearly as effective. Ammonium bicarbonate, on the other hand, had no inhibiting effect at 1·3 c.c. or 2·6 c.c. Where the alkalinity is due to potassium and ammonium bicarbonates in equal amounts (4), the reduced growth may be attributed chiefly to the potassium bicarbonate.

It should be noted that pH = 8·6 is the highest value obtainable by the addition of potassium bicarbonate to the medium, for this is the pH value of a solution of the bicarbonate alone.

TABLE X.

	Treatment of R.S./100.	*pH.*	*Botrytis Growth.*
(1)	KOH	8·4	9·4
(2)	1·3 c.c. $KHCO_3$ molecular solution per 100 c.c.	8·0	0·52
(3)	1·3 c.c. NH_4HCO_3 molecular solution per 100 c.c.	8·0	as control.
(4)	0·6 c.c. $KHCO_3$ solution 0·6 c.c. NH_4HCO_3 solution per 100 c.c.	8·0	2·09
(5)	2·6 c.c. $KHCO_3$ solution per 100 c.c.	8·4	0
(6)	2·6 c.c. NH_4HCO_3 solution per 100 c.c.	8·2	as control.
(7)	5·2 c.c. $KHCO_3$ solution per 100 c.c.	8·6	0
(8)	10·4 c.c. $KHCO_3$ solution per 100 c.c.	8·6	0
(9)	2·6 c.c. M/2 $NaHCO_3$ solution per 100 c.c.	—	0·80
(10)	5·2 c.c. M/2 $NaHCO_3$ solution per 100 c.c.	—	0
(11)	10·4 c.c. M/2N $aHCO_3$ solution per 100 c.c.		0
(12)	20·8 c.c. M/2 $NaHCO_3$ solution per 100 c.c.	—	0

Now solid ammonium carbonate, from which the molecular solution was made up, is an unstable substance which smells strongly of ammonia and is said to contain varying quantities of ammonium carbonate and carbamate, so that the use of the solid was abandoned. Evidence of the inhibiting power of ammonium bicarbonate is given above in Table VIII. However, from the nature of the medium used, it seems unlikely that ammonium bicarbonate is formed to any extent; in any case the formation of potassium bicarbonate, a much more potent growth inhibitor, would precede it.

In the work described above, the spores of *Botrytis cinerea* were always used in growth tests, but it was found that the spores of other fungi are also susceptible to the presence of potassium bicarbonate. In those fungi tested, the growth could not be readily measured by means of a micrometer eyepiece; the great differences which were observed between growth in the controls and in drops of R.S./100 containing 2·6 c.c. per cent. potassium bicarbonate are described in the table below.

TABLE XI.

The Effect of Bicarbonate on the Growth of the Spores of Various Fungi.

		Growth in about 17 Hours.			
Fungus.	*Temperature.*	*R.S./100* $pH = 4·6$	*R.S./100* $+KOH$ $pH = 8·4$	*R.S./100* $+ 2·6$ % $KHCO_3$ $pH = 8·4$	*R.S./100* $+5·2$ % $KHCO_3$
Botrytis cinerea	15°	Long, branched	Long, branched	0	
Penicillium, sp.	15°	1–2 (20 % germination)	0 + +	0	
,, ,,	20°	5–10 (20 % ,,)	as R.S./100	0	
,, ,,	25°	10 (20 % ,,)	as R.S./100	0	
Aspergillus niger	20°	0	0	0	
,, ,,	25°	Long (75 % ,,)	as R.S./100	0	
,, ,,	30°	Long, branched	Long, branched	0	
Monilia fructigena	15°	Long, branched	Long	0	
Fusarium, sp.	15°	Good	Good	v. slight	v. slight.
Mucor racemosus	15°	Good	Good	v. occasional	0

Thus, the inhibiting effect on fungal growth of potassium bicarbonate seems to be general. Further work will, it is expected, extend this list.

So much for the power of potassium bicarbonate as a growth inhibitor. To complete the story, the quantities of bicarbonate added in the above experiments must be compared with those determined in the carbonate estimations. From these estimations it was found that 0·0655 grm. is the average amount of CO_2 present as bicarbonate in 100 c.c. of the stale medium. But 1·11 c.c. molecular solution of potassium bicarbonate (see Table IX), which shifts the pH of 100 c.c. R.S./100 from 7·0 to 8·4 and practically stops growth, contains only 0·0244 grm. CO_2, that is, less than half the amount usually present in a stale medium. Thus potassium bicarbonate is in all probability formed in Richards' solution in an amount more than sufficient to inhibit growth.

It is of interest to note that these quantities of carbon dioxide as bicarbonate compare well with the amounts of free carbon dioxide which Brown (1) found to inhibit the growth of fungal spores. He showed that germination and growth of *Botrytis* spores were practically stopped in an atmosphere containing 20 per cent. CO_2 by volume, and absent in an atmosphere of 30 per cent. CO_2. Now 20 per cent. CO_2 by volume is equivalent to approximately 0·0368 per cent. grm. CO_2 at 18° C. and 30 per cent. to 0·055 per cent. grm. CO_2, which are of the same order of magnitude as the figures obtained in this work, viz. 0·0244 per cent. CO_2 and 0·0655 per cent. CO_2.

DISCUSSION.

A discussion of the problem of staling in the light of the foregoing experimental results must be prefaced by a general consideration of the phenomenon. There has been a certain amount of controversy on this question: on the one side staling has been ascribed to exhaustion of food or of some essential food element, and on the other to the inhibitory nature of metabolic products. It will be seen that this can be attributed to the two methods of experimentation available, and depends on the precise meaning attached to the word 'staling'. The problem has been attacked either by a study of the amount of fungus material produced or by determination of the germinative capacity of the medium in which a fungus has grown, the latter method being the one used in the present paper.

Several workers have used the dry-weight time curves as a basis of argument. According to them, the fungus culture may be defined as 'stale' when no further increase of mycelium takes place. Now the rate of increase of fungus material depends on two processes, the rate of uptake of nutrient and the rate of katabolic processes, and is equal to the difference between these two rates. The food supply will diminish with time, and consequently the former will decrease. The nature of the latter process is not so clear, but certainly must consist of at least two parts—respiration, as evidenced by steady evolution of carbon dioxide, and autolysis. Autolysis, that is digestion of the fungal tissues, has been shown by Dox and Maynard (2) to increase as the carbohydrate in the medium is exhausted: it results in the restoration to the medium of a large part of the nitrogen previously assimilated by the fungus until, in seven or eight weeks, equilibrium is established and the nitrogen appears to remain constant. Autolysis takes place in the older parts of the hyphae, so that the fungal weft loses its firmness and becomes soft and rather transparent. Examined under the microscope such hyphae are seen to be filled with protoplasm only at the tips: farther back the protoplasm has become very attenuated. At a certain stage these two processes exactly balance, so that there is no longer any increase in dry weight and the curve becomes flat; after this the curve declines and there is a steady diminution of weight, for autolysis is then the ruling process.

At the flattened crest of the curve, workers on these lines would say that the cultures are 'stale'. But when the condition of steady weight is reached, it does not mean that the fungus is unable to grow. The hyphal tips are still growing, but only sufficiently to make up for loss in fungal mass due to autolysis. Food is by no means exhausted, though there is insufficient to make good the wastage. Thus a culture which is stale as measured by the weight method is not yet stale when measured by active germinative capacity. Such 'staling', i.e. the point when the culture is no longer gaining weight, is necessarily delayed by the addition of food. The curve goes up once more, and hence one could justly say that staling is due in this case to exhaustion of food.

But staling, if measured by inhibition of growing capacity, will, in general, be due to the presence of inhibiting substances, for fungal hyphae will respond, as has been shown, to extremely dilute concentrations of food materials. Even when this stage is reached a certain amount of all the food elements remains: the results of dilution of the stale medium show that inhibition of growth is not due to lack of food.

The degree to which the two staling factors, exhaustion of food and concentration of staling substances, act will, with either method of measurement, vary from fungus to fungus, and even with the same fungus on different media. A weakly staling combination (fungus + particular medium) will be one in which a great part of the food supply is exhausted before an inhibitory concentration of metabolic products is reached. A strongly staling combination will be one in which staling substances rapidly accumulate, so that a large amount of unconverted food is left when the maximum dry weight is reached. Thus in the first case exhaustion of food is relatively a more important factor than in the latter. In the former case, any addition of food would produce relatively greater response: in the latter case changes in food amount would make relatively little difference.

An experiment which was devised with the object of determining to what extent each of these two factors cause reduction in growth rate of the fungus (*Fusarium*, sp.) may fittingly be described here, for it illustrates some of the points brought forward above.

Three series, of twenty-five flasks each, were set up, each flask containing 50 c.c. of R.S./10. The dilute medium was used so as to reduce the time taken by the experiment. A was a control series containing R.S./10. In B series weighed amounts of colloidal clay ('Collosan') were added to the medium in each flask. It was assumed that in B the only factor operating would be exhaustion of food, any growth-inhibiting substances being absorbed by the clay. In series C the food supply was periodically replenished by the addition of 1 c.c. of R.S. to each flask per week. Here it was assumed that the effect alone of the accumulation of growth inhibitors would be manifest. In the control both of these factors would influence growth.

Preliminary determinations of the dry weights of mycelium had been carried out in independent series of R.S. and R.S./10. Some difficulty was experienced in getting the weighings absolutely constant, and it was found that quite as satisfactory results (judged by comparison of their probable errors) were obtained if both weighing bottle + filter-paper and weighing bottle + filter + mycelium were dried at 60° C. for a standard length of time (14 days) and weighed once only. It also appeared that the results were sufficiently good if the mean of five cultures were taken for each dry weight determination.

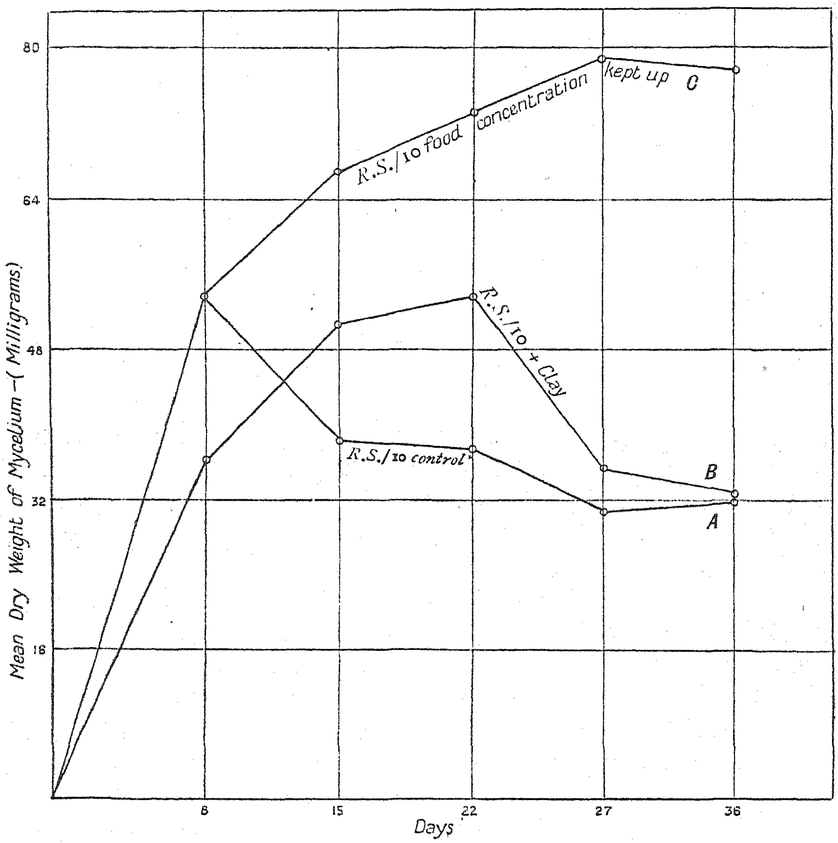
Five flasks, then, of each series, A, B, and C, were taken at intervals of one week and the mycelium (and clay) filtered off through a weighed filter-paper, dried, and the weight of mycelium determined.

The probable errors of the mean of each five weights and the percentage probable errors were calculated. The results are given in Table XII, and plotted in the figure on p. 612.

TABLE XII.

Days old.	*Medium.*	*Mean Dry Weight.*	*P.E. (%)*
		grm.	
8	A	0·0534	± 6·18
,,	B and C	0·0362	± 16·85
15	A	0·03825	± 1·844
,,	B	0·0503	± 4·745
,,	C	0·0664	± 4·985
22	A	0·0375	± 2·32
,,	B	0·0532	± 1·452
,,	C	0·0730	± 2·388
29	A	0·0309	± 5·596
,,	B	0·0354	± 6·502
,,	C	0·0784	± 2·547
36	A	0·0316	± 3·993
,,	B	0·0326	± 6·509
,,	C	0·0776	± 2·429

It will be seen that where the concentration of food is kept up, C, growth is not maintained at the initial rate, but gradually slows down and finally ceases (about 29th day). In this case the effect is due to the accumulation of metabolic products. Where colloidal clay is added cessation of growth does not occur until about the 22nd day, considerably later than in the control (8th day), though earlier than in C. Here B, the chief factor operating, may be taken to be exhaustion of food. After attaining a maximum, the curve begins to decline, which suggests that autolysis of the mycelium is taking place. Autolysis is shown (see figure above) in the control curve A, but there is no sign of it in C, where the food supply is kept up. The effect of autolysis, as well as of diminution of nutrient, will explain the fact that the maximum in B is not so high as that in C. The conclusion, then, seems justified that the two factors, exhaustion of food and accumulation of metabolic products, are here equally important in reducing the rate of growth.



It is obvious then that the controversy arises from the fact that the arguments are proceeding from different bases. Those engaging in the controversy, indeed, never meet on the same ground. The activity of the fungus is controlled in varying degree by both exhaustion of nutrient and the accumulation of toxic products of metabolism. Determinations of the dry weight of the mycelium produced approach the question from the aspect only of the growth of the fungal colony: germination tests approach it from the more fundamental aspect of the changes brought about in the medium by that growth, which changes affect profoundly the capacity of the medium for supporting further growth.

Any value of the results described in the first part of the work (3) lies in the knowledge gained of the metabolism of *Fusarium*, so that we are now in a better position to evaluate and analyse the results of experimental work on 'staling'.

The hope that the metabolism of *Fusarium* might be such as to justify its selection as a 'typical' fungus has been realized: the metabolic products are simple and of the kind that would be expected in the degradation or fermentation of sugars. Moreover, Richards' solution has proved to be one which gives rise to no toxic chemical residues, and the results may, therefore, be taken to have a general application and cannot, as in the case of most of the work described in the historical section, be ascribed to the chemical nature of part of the food supply.

The most interesting of the metabolic products from the point of view of the study of staling are the organic acids. Three independent lines of approach, viz. ether extraction of the acidified medium, chemical analysis, and the addition to the fresh medium of various organic compounds, have led to the conclusion that these acids are produced and are active under the conditions of experimentation.

The results obtained by other workers have suggested that the final part of the 'staling principle' may be found in the liquid after it has been boiled and acidified, and that it operates in the acidified medium. As a matter of fact, as has been amply demonstrated (3), the poor growth of *Botrytis* spores in an acidified stale medium is due to simple organic acids which are liberated by the acidification. These acids do not, however, represent the final part of the staling principle; a growth-inhibiting factor, which does not exist in the alkaline medium, has been shown to be introduced by the acidification.

By concentrating attention on the removal of staleness step by step we run the risk of being led in the wrong direction. The more profitable course is to consider the stale medium as a whole, that is, in its alkaline condition. In this way the definite conclusion has been reached that, by the accumulation in it of potassium bicarbonate, Richards' solution is unfitted to support further growth. This compound has a very marked property of inhibiting fungal growth, which cannot be ascribed to its slight alkalinity, for potassium carbonate and potash at the same pH have no effect on growth. Potassium bicarbonate is a much more potent growth inhibitor than any of the other compounds demonstrated in the stale medium.

The interest of this discovery lies in its universal application; for it is reasonable to suppose that all fungi are capable of forming bicarbonates by the combination of the carbon dioxide of their own respiration with the constituents of the medium, or with the residues thereof after part has been assimilated, e.g. potassium after the nitrate radical is assimilated. It is, then, suggested that the growth of fungi on artificial media, and probably also on natural media, is, in general, stopped by the accumulation of bicarbonates (usually potassium bicarbonate) whenever the medium is such that assimilation sets free a basic radical.

SUMMARY.

Organic acid radicals do not reduce the growth of *Botrytis* spores if the medium be made alkaline. They cannot, therefore, act as staling agents in an alkaline stale medium.

The nitrogen source, potassium nitrate, is the origin of alkalinity, as is shown by the results of omission in turn of the constituents of Richards' solution. Dilution of the nitrogen source causes the pH to change more slowly.

Estimations show that a considerable amount of bicarbonate and a small amount of carbonate are present in the stale medium. The presence of bicarbonate explains the fact that boiling the stale medium increases its alkalinity.

A study of the effect on the growth of fungal spores of the alkaline metabolic products has led to the conclusion that the small quantities of ammonia produced are probably ineffective, but that bicarbonates, potassium, and, in a lesser degree, ammonium are capable of inhibiting *Botrytis* growth when present in amounts even smaller than those found in the stale medium. In a medium of pH = 8·2 produced by potassium bicarbonate growth is completely inhibited; potassium carbonate and hydroxide have no sensible effect at this pH. The loss of germinative capacity of the stale Richards' solution is attributed to accumulation of potassium bicarbonate.

In addition to *Botrytis cinerea*, other fungi, of which a list is given, are affected by potassium bicarbonate.

The problem of 'staling' is discussed, and the value of the evidence obtained from dry-weight time curves and from the germinative capacity of the medium is considered.

The results of the work are reviewed, and it is suggested that 'staling'

is, in general, due to the formation of bicarbonate by the carbon dioxide of respiration whenever the medium is such that a basic radical is set free.

I wish to place on record here, first my gratitude to Professor V. H. Blackman, who has placed at my disposal the facilities of his department, for his interest in the work, embodied in this and the previous paper, and, secondly, my indebtedness to Assistant Professor W. Brown for valuable criticism and suggestion.

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The Absorption of Salts by Storage Tissues.

BY

WALTER STILES.

With four Figures in the Text.

INTRODUCTION.

THE prevailing ideas of the mechanism of the intake of salts by plant tissue were, until comparatively recently, characterized by a charming simplicity. This was largely due to the very general assumption of what I have elsewhere called the 'simple osmotic view' of the cell (9). The passage of a salt into a cell was supposed to depend in the first instance on the capacity of the salt to pass through a plasmatic membrane surrounding the protoplasm. Similarly, the permeability of a membrane surrounding the vacuole determined whether the salt, having reached the protoplasm, would penetrate into the vacuole. If the salt were capable of passing through the plasmatic membrane various things might happen. The salt might not react with any constituent of the protoplasm, nor, if it passed the inner plasmatic membrane, with any constituent of the vacuole, in which case absorption would continue until there were equality of concentration within and without the cell. On the other hand, a reaction might take place between a penetrating salt and a cell constituent, in which case, as the salt itself would be removed as it reached the protoplasm or vacuole, as the case might be, absorption would continue much farther than would be necessary to bring about equality of concentration within and without the cell if there were no such reaction. Thus the great absorption of methylene blue and other dyes by living cells has been explained by supposing that the dyes react with tannins inside the cell, so that compounds incapable of diffusing through the cell membranes are produced.

There have been accumulating during recent years, however, as a result of experimental work, indications of the inadequacy of this simple view of salt intake by plant tissue. Facts are suggested, which, if actually

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existent, indicate the necessity for the abandonment, or very considerable modification, of this simple view. These suggested facts are:

1. The constituent ions of a salt are not necessarily absorbed in equivalent quantities by plant tissue; that is, the salt is not absorbed as such.

2. The quantity of a salt, or ion of a salt, relative to the concentration, that is absorbed by a plant cell is dependent on the concentration; the more dilute the solution the greater the relative quantity of salt absorbed from the solution. Thus, when the cell is immersed in a comparatively strong solution equilibrium may be reached long before sufficient salt or ion has been absorbed to bring about equality of concentration within and without the cell, while in dilute solutions the quantity of salt absorbed may be many times that which would be necessary to bring about equality of concentration within and without the cell.

For the first of these propositions there now exists a considerable amount of evidence obtained by means of direct analysis of the solutions in which living plants or living plant tissues have been immersed. The bulk of these observations have been made on plants, the roots of which were surrounded by the experimental solutions. A few observations have also been made on storage tissues, such as beetroot and *Dahlia* tuber, and also on a number of lower plants, such as *Codium* among the algae and yeast among saprophytes. The available data have recently been summarized by the present writer (10), and they will therefore not be repeated here.

The mechanism of this unequal absorption of ions is less clear. There appear to be two possible consequences. One is that the excess of the more absorbed ion is accompanied into the tissue by a quantity of one of the constituent ions of water, hydrogen or hydroxyl, whichever is of opposite sign to the more absorbed ion. In this case the liquid external to the tissue will be left either alkaline or acid. Thus if tissue is immersed in a solution of sodium chloride and the sodium ion is absorbed in excess of the chlorine ion, the excess of sodium ion would be accompanied into the tissue by an equivalent quantity of hydroxyl ion, leaving the same equivalent quantity of hydrogen ion to balance the excess of chlorine ion remaining in the external solution. Put in another way, part of the sodium enters the tissue as sodium hydroxide and the external solution comes to contain a certain amount of ionized hydrochloric acid. If, on the other hand, the chlorine ion is absorbed in excess, hydrogen ion accompanies the excess of chlorine ion into the tissue and the external solution becomes alkaline.

The acidity and alkalinity said to be developed in some water-culture solutions have been ascribed in recent years to this possible consequence of the unequal absorption of ions (cf. 4).

The second possibility is that excess of the more absorbed ion is replaced in the external solution by some other ion or ions of the same sign diffusing out from the tissue. Thus, using again the example of sodium chloride, the excess of sodium absorbed might be compensated by the diffusion out from the tissue of magnesium, potassium, or other kation or kations; or, if chlorine is the more absorbed ion, by the diffusion out of nitrate, citrate, malate, or other anion or anions. Cases in which such an interchange of ions is supposed to take place are on record (6).

The evidence for the second possibility, the dependence of the position of equilibrium in salt absorption on the concentration, is derived chiefly from the work of Stiles and Kidd (11, 12), who obtained evidence to the effect that the position of equilibrium in salt intake by storage tissues is given by the equation

$$y = kc^m,$$

where y is the final internal concentration, c the final external concentration, and k and m are constants.

This means that the ratio of the internal to the external concentration at equilibrium, the 'absorption ratio', increases as the concentration diminishes. It was found that the absorption ratio might range from only a fraction of, to many times, unity, the actual value depending on the nature of the salt, the concentration, and probably on the nature of the tissues.

While, for reasons given in the papers cited, it is highly probable that these conclusions, drawn from measurements of the electrical conductivity of the external solution, are valid, it must be admitted that unequivocal results can only be obtained by actual chemical analysis, and it is highly desirable that the results should be confirmed or disproved by means of chemical analysis.

In the work described in the present paper special attention has been directed to the questions noted above. The experiments, the results of which are recorded here, have been made during the last four years with the object of establishing, by means of chemical analysis supplemented by physico-chemical methods, a complete view of the relations in regard to salt intake and excretion when storage tissues are immersed in a solution of a single salt. The problem, so stated, appears alluringly simple, but its experimental solution is somewhat laborious, largely on account of difficulties attending the quantitative analysis of dilute solutions which may contain quantities of organic material derived from the absorbing tissues.

The problem resolves itself into several parts. These are (1) the unequal absorption of the constituent ions of a salt, (2) the course of absorption of the ions, and (3) the position of equilibrium reached in the intake. These various aspects of the problem will be dealt with consecutively in later sections of the paper.

EXPERIMENTAL METHODS.

A variety of storage tissues have been employed during the investigation, including the roots of red beet, carrot, parsnip and turnip, the rootstock of white bryony (*Bryonia dioica*), and tubers of Jerusalem artichoke (*Helianthus tuberosus*). The tissues were used in the form of discs, 2 mm. in diameter and 0·1 mm. in thickness, cut by means of a hand microtome from cylinders of the tissue cut out from the storage organs by means of a cork-borer. After cutting, the discs were washed for one or two days in running tap water, then in several changes of distilled water, in order to remove material from the cut surface cells. Usually 40 discs were used in 100 c.c. of solution, and where it was expedient to depart from this quantity of solution the same proportion of material to solution was maintained. Immediately before transference to the experimental solution each batch of discs was dried between filter-paper and weighed. At the end of an experiment the batch of discs was again weighed. The maintenance of turgor, as shown by the retention of the original weight, is sufficient evidence that the vitality of the tissue is unimpaired by the experimental treatment.

Experiments were conducted at 20° C. or in the neighbourhood of this temperature. The bottles containing discs and solution were kept in a water bath maintained at constant temperature by means of a gas-mercury-toluene thermo-regulator. In most experiments the discs were kept in movement by shaking the bottles in a specially constructed trolley driven backwards and forwards through the water by means of a motor. In a few experiments the discs were kept still.

Solutions of single salts only were employed in the series of experiments here described. The salts used were ammonium chloride, ammonium sulphate, ammonium phosphate, potassium chloride, sodium chloride, and sodium sulphate. Ammonium was estimated by boiling with alkali and estimating the ammonia driven off in the usual way; potassium was estimated as perchlorate, sodium as pyroantimonate (2), chloride by titration with silver nitrate, sulphate as barium sulphate, and phosphate by means of uranium acetate.

Electrical conductivity measurements were made by Kohlrausch's method with use of a dipping electrode, and determinations of hydrogen-ion concentration were made electrometrically by means of the calomel electrode and a vernier potentiometer made by Tinsley, and also by the indicator method.

THE UNEQUAL ABSORPTION OF THE IONS OF A SALT.

Solutions of the salts in which discs of the various storage tissues were immersed were analysed after absorption had been allowed to proceed for times varying from one hour to three days. The results obtained leave no doubt that the unequal absorption of the constituent ions of a salt is a very general phenomenon. A selection of very typical results is given in Table I. Of the eleven sets of analyses there shown, only in two cases was equality of concentration approached, while in some cases the divergence from equality was very considerable. This was notably the case with ammonium chloride, ammonium phosphate, and sodium chloride. The few observations of Meurer (3) and Ruhland (8) indicating the inequality of absorption of the two ions of a salt by storage tissues are thus confirmed, although the numbers themselves do not agree well with those given above. However, it seems reasonable to suppose that the unequal absorption of the constituent ions of a salt by storage tissue is a general phenomenon.

TABLE I.

The Unequal Absorption of the Constituent Ions of a Single Salt by Storage Tissue.

Salt.	*Conc. of Salt in Normalities.*	*Tissue.*	*Duration of Experiment in Hours.*	*Absorption in Percent. of Original Amount.*	
				Kation.	*Anion.*
Ammonium chloride	0·01	Carrot	48·0	57·2	32·2
Ammonium phosphate	0·02	,,	23·5	28·85	6·0
,, ,,	,,	,,	27·5	34·1	6·95
,, ,,	,,	Parsnip	24·33	34·5	8·2
,, ,,	,,	Beetroot	24·5	19·6	5·1
Ammonium sulphate	0·1	Carrot	22·0	3·9	4·2
Potassium chloride	,,	,,	22·0	4·6	6·3
Sodium chloride	,,	Artichoke	22·6	5·4	1·9
,, ,,	,,	Carrot	47·1	18·5	8·5
,, ,,	,,	Bryony	50·2	21·0	8·4
Sodium sulphate	,,	Carrot	22·0	15·0	8·0

TABLE II.

The Influence of Concentration on the Unequal Absorption of the Constituent Ions of a Single Salt by Carrot Root.

Initial Concentration of Solution in Normalities.	*Ammonium Chloride. Percentage Absorption in 48 Hours.*		*Sodium Chloride. Percentage Absorption in 48 Hours.*	
	Kation.	*Anion.*	*Kation.*	*Anion.*
0·1	12·9	5·9	25·2	8·3
0·01	57·2	32·2	36·1	15·8
0·001	97·1	80·7	38·1	22·8

Influence of Concentration on the Unequal Absorption of Ions. In experiments with whole plants, Miss Redfern (6) found that roots of pea and maize absorbed the two ions of calcium chloride to unequal extents, but that the inequality was lessened with decreasing concentration. The influence of concentration was examined for carrot tissue in the case of two salts, ammonium chloride and sodium chloride. Three concentrations were employed in each case, namely, 0·1 N., 0·01 N., and 0·001 N. The results obtained are summarized in Table II. With both salts the absorption of the two ions certainly did approach nearer equality the more dilute the solution, the effect being particularly noticeable with the most dilute solution employed.

As far as these results go it would thus appear that storage tissue behaves similarly to living roots, and that the divergence between the extent of absorption of the two ions of a salt is lessened with dilution of the salt.

The Maintenance of Equivalent Quantities of Kations and Anions in the External Solution. The changes in ionic composition of the external solution involved in the unequal absorption of the two ions of a salt have been examined in the case of sodium chloride. It has been pointed out that maintenance of equivalent quantities of positively and negatively charged ions could be brought about either by the solution becoming acid or alkaline, or by the diffusion of ions out from the tissue. Determinations of hydrogen-ion concentration of the external solutions showed that the latter do not depart appreciably from neutrality throughout the course of absorption. It would, therefore, appear that the excess of the more absorbed ion must be replaced in the external solution by diffusion out from the tissue of similarly charged ions. This was found to be the case. Carrot tissue was immersed in 0·1 N. solution of sodium chloride for forty-seven hours. Analysis of this solution, after removal of the tissue, showed that it was 0·092 N. in regard to chloride and 0·075 N. in regard to sodium, an excess concentration of chloride of 0·017 N. But the solution was now 0·009 N. in regard to calcium and 0·003 N. in regard to potassium; a trace of magnesium was also present. The calcium, potassium, and magnesium ions go a long way towards balancing the excess of chlorions over sodium ions. That they do not quite balance may be due to the presence of other kations or to experimental error, which, on account of the small quantities of solution available for analysis, may be considerable. So far, then, as these experiments go, it may be concluded that the maintenance of equivalent quantities of kations and anions in the external solution is brought about by diffusion of ions out from the tissue.

THE COURSE OF ABSORPTION OF IONS.

The course of absorption of ions by storage tissues has been examined in the cases of carrot, parsnip, and beetroot in 0·02 N. ammonium phosphate, and of artichoke tuber and carrot in 0·1 N. sodium chloride. In some cases measurements of the electrical conductance of the solutions were made as well as chemical analyses.

Carrot in 0·02 N. Ammonium Phosphate. Two series of experiments were performed at 20° C. Determinations were made of the electrical conductance and of the concentration of ammonium and phosphate in the solutions, after absorption had proceeded for various times up to two days. The results of one series are given in Table III; the results obtained in the other were very similar and confirmatory. It will be observed that the absorption of both ammonium and phosphate is relatively rapid at first, but that after four or five hours the rate of absorption rapidly declines. The rate of absorption of phosphate is throughout much smaller than that of ammonium. Absorption, as measured by conductance, is throughout intermediate between that of ammonium and phosphate, but approximates more nearly to that of phosphate than of ammonium. For the sake of clearness the results are shown graphically in Fig. 1.

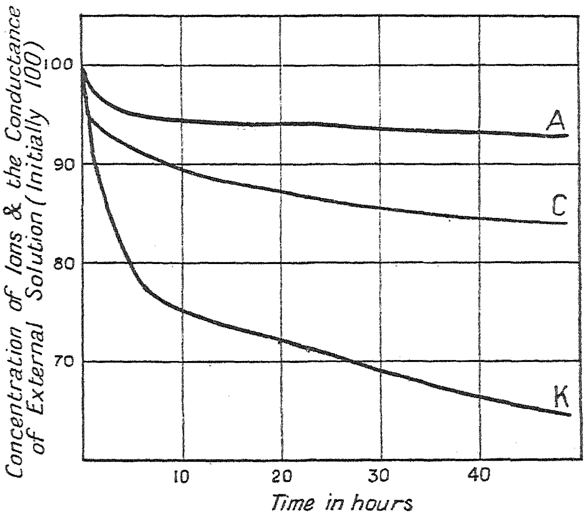

FIG. 1. Curves to illustrate the absorption by carrot of the ions of ammonium phosphate from a 0·01 N. solution. (K, kation; A, anion; C, conductance.)

TABLE III.

Absorption of Ammonium and Phosphate from 0·02 N. Ammonium Phosphate by Carrot Root.

Time in Hours.	*Concentration of Ammonium in External Solution in Percent. of Initial Concentration.*	*Concentration of Phosphate in External Solution in Percent. of Initial Concentration.*	*Electrical Conductance of External Solution in Percent. of Initial Conductance.*
0·0	100	100	100
1·0	92·15	97·14	94·45
5·08	78·36	94·93	91·83
23·5	71·15	93·99	86·84
49·0	64·59	92·72	83·87

Parsnip in 0·02 N Ammonium Phosphate. The results of the experiments with parsnips are summarized in Table IV and shown graphically in Fig. 2. The course of absorption of the ions and the changes in electrical conductance of the external solution are very similar to those involved with carrot, and the quantities of the ions absorbed after two days' immersion of the tissue are much the same.

TABLE IV.

Absorption of Ammonium and Phosphate from 0·02 N. Ammonium Phosphate by Parsnip Root.

Time in Hours.	*Concentration of Ammonium in External Solution in Percent. of Initial Concentration.*	*Concentration of Phosphate in External Solution in Percent. of Initial Concentration.*	*Electrical Conductance of External Solution in Percent. of Initial Conductance.*
0·0	100	100	100
1·0	92·34	97·59	91·96
5·32	74·33	94·52	85·37
24·33	65·52	91·77	78·94
47·0	59·39	91·09	74·29

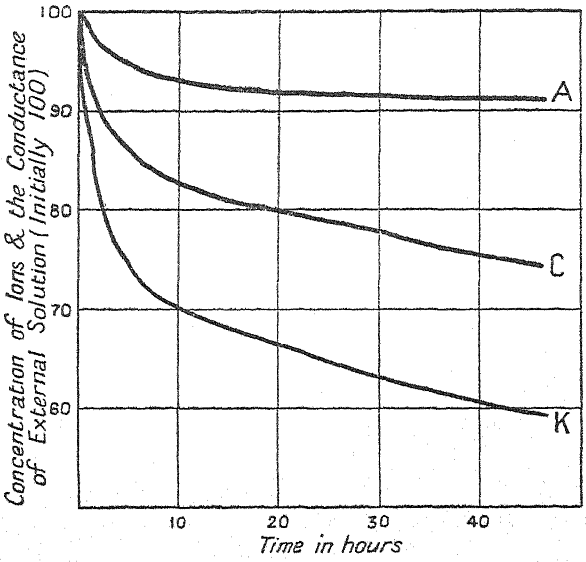

FIG. 2. Curves to illustrate the absorption by parsnip of the ions of ammonium phosphate from a 0·02 N. solution. (K, kation; A, anion; C, conductance.)

Beetroot in 0·02 N. Ammonium Phosphate. The absorption of ammonium and phosphate from ammonium phosphate solution by beetroot is indicated in Table V. The absorption of ammonium is again considerably more rapid than that of phosphate. While the general relations are the same as in the cases just described, the numbers suggest that beetroot absorbs less than parsnip and carrot, other conditions being the same.

TABLE V.

Absorption of Ammonium and Phosphate from 0·02 N. Ammonium Phosphate by Beetroot.

Time in Hours.	*Concentration of Ammonium in External Solution in Percent. of Initial Concentration.*	*Concentration of Phosphate in External Solution in Percent. of Initial Concentration.*	*Electrical Conductance of External Solution in Percent. of Initial Conductance.*
0·0	100	100	100
1·0	96·56	99·65	98·04
5·25	85·45	98·63	
24·5	80·46	94·86	
47·75	75·88	94·86	92·70

Artichoke (Helianthus tuberosus) Tuber in 0·1 N. Sodium Chloride. The absorption of the ions of sodium chloride by tuber of *Helianthus tuberosus* was found to follow the same general course as in the cases already described. The kation was again absorbed in excess of the anion, but the absorption proceeded more slowly in both cases. This is due, in part at any rate, to the use of a considerably stronger solution, but it may also be partly correlated with the nature of the salt. It is noteworthy that the percentage fall in conductance is almost identical with that of the less absorbed ion, so that in this case the change in electrical conductivity gives a very good measure of the change in concentration of the less absorbed ion. The results obtained are summarized in Table VI.

TABLE VI.

Absorption of the Ions of Sodium Chloride by Artichoke Tuber from a 0·1 N. Solution of the Salt.

Time in Hours.	*Concentration of Sodium in External Solution in Percent. of Initial Concentration.*	*Concentration of Chloride in External Solution in Percent. of Initial Concentration.*	*Electrical Conductance of External Solution in Percent. of Initial Conductance.*
0·0	100	100	100
8·7	97·475	98·5	98·15
22·575	94·61	98·1	98·3
46·6	91·1	97·7	97·7

Carrot in 0·1 N. Sodium Chloride. The results obtained in a series of experiments extending over five days are summarized in Table VII. The results are very similar to those obtained with artichoke, the quantities of kation and anion absorbed in two days being practically the same as the quantities absorbed by artichoke tuber. It is very interesting to note that most of the absorption takes place during the first twenty-four hours, and that after two days' immersion the further intake of the ions proceeds very slowly.

TABLE VII.

Absorption of the Ions of Sodium Chloride by Carrot from a 0·1 N. Solution of the Salt.

Time in Hours.	*Concentration of Sodium in External Solution in Percent. of Initial Concentration.*	*Concentration of Chloride in External Solution in Percent. of Initial Concentration.*
0·0	100	100
24·0	92·25	97·85
47·5	91·6	97·2
72·0	90·1	96·65
125·7	89·75	96·5

The results here recorded, as well as those of some experiments with potassium chloride and calcium chloride in which electrical conductance and absorption of chloride were alone measured, confirm on the whole the conclusions drawn previously by Stiles and Kidd (11, 12) from experiments in which change in electrical conductivity of the external solution was used as a basis for measuring the absorption of salt or of the less absorbed ion, and the experiments here described furnish evidence of the legitimacy of the conclusions drawn from the earlier experiments.

THE POSITION OF EQUILIBRIUM REACHED IN ION INTAKE.

One of the most important of the conclusions reached by Stiles and Kidd was that the absorption of a salt or its ions does not proceed to a position of equilibrium in which there is equality of concentration within and without the cell, but that the position of equilibrium depends on the nature of the salt and on the concentration, the lower the concentration the higher being the ratio of the final apparent internal concentration to the final external concentration, this ratio varying from values less than unity in the case of high concentrations to values many times unity in low concentrations.

The question has been reinvestigated by chemical analysis. In most cases the absorption ratio was calculated from the internal and external concentrations after absorption had been allowed to proceed for two days.

As indicated in the preceding section of this paper, absorption does proceed after this time, but very slowly, so that the values obtained for the absorption ratios are actually a little less than the absorption ratios at equilibrium. The absorption ratio in any case is an approximate value, as it is assumed that the absorbed ion is distributed throughout the whole of the tissue except the injured surface cells,[1] whereas the absorbed ion may actually be accumulated to different degrees in cell-wall, protoplasm, and vacuole, each of which may, and probably does, have its own particular absorption ratio.

The experiments described in preceding sections of this paper show that the absorption ratios when equilibrium is approached are far removed from unity in the majority of cases. The values obtained for the absorption ratios in a number of these experiments are recorded in Table VIII.

TABLE VIII.

Absorption Ratios.

Salt and Duration of Absorption.	*Tissue.*	*Ion.*	*Concentrations in Normalities.*			*Absorption Ratio.*
			Initial External.	*Final External.*	*Final Internal.*	
Ammonium phosphate 49 hours	Carrot	NH_4	$\frac{2}{3}$(0·02)	$\frac{2}{3}$(0·0129)	$\frac{2}{3}$(0·0628)	4·87
		PO_4	0·02	0·0185	0·0133	0·72
Ammonium phosphate 47 hours	Carrot	NH_4	$\frac{2}{3}$(0·02)	$\frac{2}{3}$(0·0123)	$\frac{2}{3}$(0·0681)	5·54
		PO_4	0·02	0·0184	0·0142	0·77
Ammonium phosphate 47 hours	Parsnip	NH_4	$\frac{2}{3}$(0·02)	$\frac{2}{3}$(0·0119)	$\frac{2}{3}$(0·0717)	6·03
		PO_4	0·02	0·0182	0·0159	0·87
Sodium chloride 46·6 hours	Artichoke	Na	0·1	0·0911	0·0788	0·86
		Cl	0·1	0·0977	0·0204	0·21
Sodium chloride 46·5 hours	Carrot	Na	0·1	0·0895	0·0929	1·04
		Cl	0·1	0·0955	0·0398	0·42
Sodium sulphate 22 hours	Carrot	Na	0·1	0·0850	0·133	1·56
		SO_4	0·1	0·0957	0·0381	0·40
Ammonium sulphate 22 hours	Carrot	NH_4	0·1	0·0961	0·0345	0·36
		SO_4	0·1	0·09575	0·0376	0·39
Potassium chloride 22 hours	Carrot	K	0·1	0·0954	0·0407	0·43
		Cl	0·1	0·0937	0·0558	0·60

It is clear from these results that the absorption ratio at the approach of equilibrium is only exceptionally in the neighbourhood of unity. Moreover, if there were any serious error in computing the volume of the tissue for calculation of the final internal concentration, the wide range of values for the absorption ratio is sufficient to show that this ratio is most usually something other than unity.

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[1] Allowing for the cut surface cells the volume of tissue employed in an experiment with forty discs is approximately 11·3 c.c.

The Influence of Concentration on the Position of Equilibrium. As already mentioned, earlier work with the indirect electrical conductivity method indicated that the absorption ratio increases with dilution. In the present investigation this question was examined in the cases of carrot immersed in sodium chloride and ammonium chloride. The concentrations employed of each of these salts were 0·1 N., 0·01 N., and 0·001 N., and absorption was allowed to proceed for about forty-eight hours. The results obtained are shown in Table IX, and exhibit clearly how the absorption ratio in the case of both ions of the salts increases with dilution. This is particularly noticeable with ammonium chloride, 13 per cent. of the ammonium of which is absorbed in forty-eight hours from a 0·1 N. solution, 57 per cent. from a 0·01 N. solution, and 97 per cent. from a 0·001 N. solution. The results with the anion are equally striking.

TABLE IX.

Influence of Concentration on the Position of Equilibrium attained in the Absorption of the Constituent Ions of Sodium Chloride and Ammonium Chloride by Carrot Root.

Salt.	*Initial Concentration in Normalities.*	*Final Concentration of Kation in Normalities.*		*Final Concentration of Anion in Normalities.*		*Absorption Ratio.*	
		External.	*Internal.*	*External.*	*Internal.*	*Kation.*	*Anion.*
Sodium chloride	0·1	0·0748	0·223	0·0917	0·07345	2·98	0·801
	0·01	0·00639	0·03195	0·00842	0·0140	5·00	1·66
	0.001	0·000619	0·00337	0·000772	0·00202	5·45	2·62
Ammonium chloride	0·1	0·0871	0·1141	0·0941	0·0520	1·31	0·553
	0·01	0·00428	0·0506	0·00678	0·0285	11·8	4·20
	0·001	0·0000293	0·00859	0·0001923	0·00715	293·2	37·2

Although the increase in the absorption ratio with dilution in the case of sodium chloride is perfectly definite, the increase of the ratio with dilution is not nearly so striking as in the case of ammonium chloride. Indeed, the absorption ratios found here for the more dilute solutions of sodium chloride are very much lower than those obtained by the electrical conductivity method. This might be due to unsuspected sources of error in the latter method, and it was observed in the present experiments that in these solutions there is a considerable exosmosis of organic material, which would, of course, depress the conductivity significantly in the diluter solutions, and might be sufficient to account for the different results obtained by the direct method of chemical analysis and the indirect method of electrical conductivity measurement. On the other hand, the different results may be correlated with differences in the varieties or the previous histories of the roots used in the two series of experiments. It is not possible to speak definitely on the influence of previous history of the tissue at this time, but investigations on this question are at present in progress.

It was earlier concluded (11) from conductivity measurements that the relation between final internal and final external concentration is given approximately by the equation,

$$y = kc^m \quad \text{or} \; \log y = m \log c + k,$$

where y is the final internal concentration, c the final external concentration, and k and m are constants. The relation between the final internal and final external concentrations in the case of the ions of sodium chloride are shown graphically in Fig. 3, where the logarithms of these two values are plotted against one another. The values for ammonium chloride are similarly plotted in Fig. 4. It will be observed that the relation is approximately a linear one, so that the absorption of the ions is given fairly approximately by the equation cited above. Actually in all cases, whereas the absorption is approximately given by the equation, there is a slight divergence from it, the absorption in higher concentrations being somewhat lower (or in lower concentrations somewhat higher) than that required by the equation.

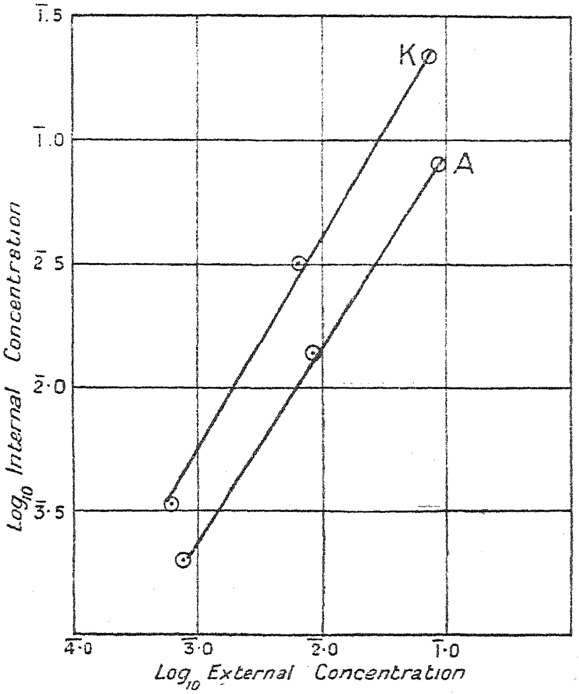

FIG. 3. · The relation between final internal and external concentrations in the absorption of sodium chloride by carrot. (K, kation; A, anion.)

It is interesting to note that this relation between concentration and the position of equilibrium in absorption is not confined to inorganic salts, as a similar increase in the value of the absorption ratio with dilution has also been observed by Miss Redfern (7) with a number of dyes, in some cases of which the same equation $y = kc^m$ holds approximately. Also increased relative absorption is not confined to storage tissues, for it has been observed by the present writer in a number of unpublished observations on absorption by seeds, and has also been recently recorded by Hevesy (1) for absorption of lead nitrate by bean roots. Thus the latter worker observed that in twenty-four hours the root of *Vicia Faba* absorbed 0·3 per cent. of the lead in 200 c.c. of a 0·1 N. solution of lead nitrate, whereas 60 per cent. of the lead was absorbed in the same time from the same volume of a 10^{-6} N. solution of the same salt, a result very similar to those recorded in this paper. The phenomenon has also been noted by Pantanelli (5).

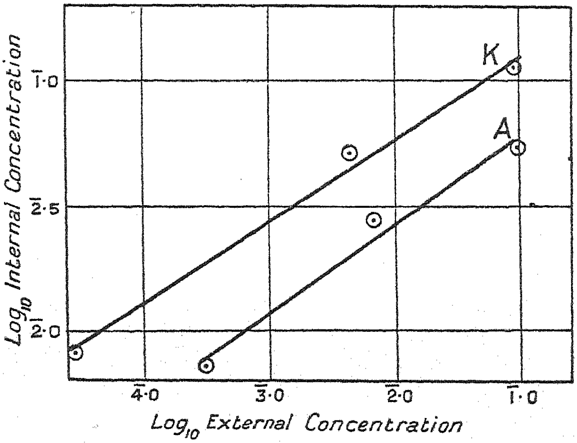

FIG. 4. The relation between final internal and external concentrations in the absorption of ammonium chloride by carrot. (K, kation ; A, anion.)

DISCUSSION.

The investigation described in this paper was undertaken in order to obtain a general view of the relation of storage tissues towards inorganic salts, and as a result the following description can be given. When storage tissue is immersed in a solution of a single salt the ions of the salt are not necessarily absorbed in equivalent quantities, the balance of the ionic charges in the external solution being maintained by the diffusion of ions out from the tissue. Absorption of either ion proceeds comparatively rapidly at first, but after four or five hours the rate of absorption falls off greatly. Absorption of each ion proceeds towards an equilibrium condition which is not that of equality of concentration of the ion within and without the tissue, but which is dependent on the concentration. The absorption ratio, that is, the ratio of the final internal concentration to the final external concentration at equilibrium, varies continuously from a fraction of unity to many times unity with decreasing concentration, the position of equilibrium being given approximately, although not exactly, by the adsorption equation.

From each of the three aspects of the problem of the absorption of salts dealt with in this paper, it is clear that the simple view of the cell largely held previously is inadequate. The unequal rate of absorption of the two ions of a salt, the fact that the absorption of the two salts does not follow a logarithmic course, and does not, therefore, obey Fick's law, and the dependence of the position of equilibrium in absorption on the concentration, all indicate that the absorption of inorganic salts by plant cells is not a simple process of diffusion through a cell membrane.

Two comparatively simple explanations suggest themselves. It might be supposed that the salt combines chemically with a cell constituent. While this would explain the high absorption ratios in the case of low concentrations of solution, it will not explain the absorption ratios below unity in the case of higher concentrations of solution. For if the salt is capable of combining with a cell constituent, it must be concluded that it is capable of entering the cell, and it would then be supposed that after all the particular cell constituent combining with the salt was utilized, absorption of the salt would continue until there was equality of concentration of the salt on the two sides of the cell membranes. The chemical combination view, therefore, also involves the assumption that the salt itself, or the compound formed by its action within the cell, renders the cell membranes impermeable to the salt, so that absorption stops before enough of the salt is taken up to bring about equality of concentration on the two sides of the membrane. The very regular relation between concentration and absorption, however, militates against the view that a cell membrane is rendered impermeable in high concentrations of the salt, while low concentrations have no such action.

The alternative simple explanation is that the salt or its ions are adsorbed at the surface of some cell constituent or constituents. It is well known that protoplasm is a colloidal system, while there is evidence that material in the colloidal condition is also present in the vacuole, so that the plant cell might well act as an adsorbent of inorganic salts. In favour of this view, now by no means a new one, is the relation between final internal and final external concentration of the absorbed salt, which is the same as that which would hold if the salt were adsorbed. There appears to be no fatal argument that can be advanced against this view, but, on the other hand, the agreement with the adsorption equation cannot be regarded as sufficiently convincing evidence in itself.

Before either of these views, or any other view, of the mechanism of the absorption of inorganic salts by storage tissues can be advanced as a theory of salt intake with any sound basis, further experimental evidence is needed, and the matter will therefore not be discussed further at this stage. From the investigations described in this paper, together with the results of earlier work alluded to in previous sections, it is clear that the view of salt intake as a process of simple diffusion through a cell membrane must be abandoned or much modified or supplemented. This is all the more important, as considerable masses of data with regard to the permeability of plant cells to salts have been obtained by the use of methods, the plasmolytic methods, which assume that salt intake is such a simple diffusion process through a protoplasmic membrane. It is evident that the results obtained with the plasmolytic methods require a very close scrutiny, and should either be confirmed by some other method or abandoned.

SUMMARY.

1. The absorption by storage tissue of an inorganic salt from its solution in water is characterized by an unequal absorption of the constituent ions of the salt. The balance between the oppositely charged ions in the external solution is maintained by the diffusion out from the tissue of ions charged similarly to the ion absorbed in excess.

2. The ions are absorbed comparatively rapidly at first, but after the first five or six hours the rate of absorption slows down considerably. Absorption is still proceeding slowly after five days in some cases, but after the expiration of the first two days the rate of absorption is very slow. Absorption does not follow a simple logarithmic course over the whole absorption period.

3. The absorption of each ion proceeds towards an equilibrium position which is not determined by equality of concentration within and without the tissue, but which is dependent on the concentration of the salt. With decreasing concentration relatively more salt is absorbed, the position of equilibrium being approximately given by the adsorption equation $y = kc^m$.

4. These findings indicate that the absorption of salt by storage tissues cannot be a simple process of diffusion of salt through a cell membrane. As this assumption underlies measurements of salt intake and of cell permeability made by the plasmolytic methods, conclusions with regard to these questions based on results obtained by plasmolytic methods should be confirmed by other methods or disregarded.

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UNIVERSITY COLLEGE, READING.
2 *November*, 1923.

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Myrsiphyllum and Asparagus: a Morphological Study.

BY

AGNES ARBER, M.A., D.Sc.

With forty-six Figures in the Text.

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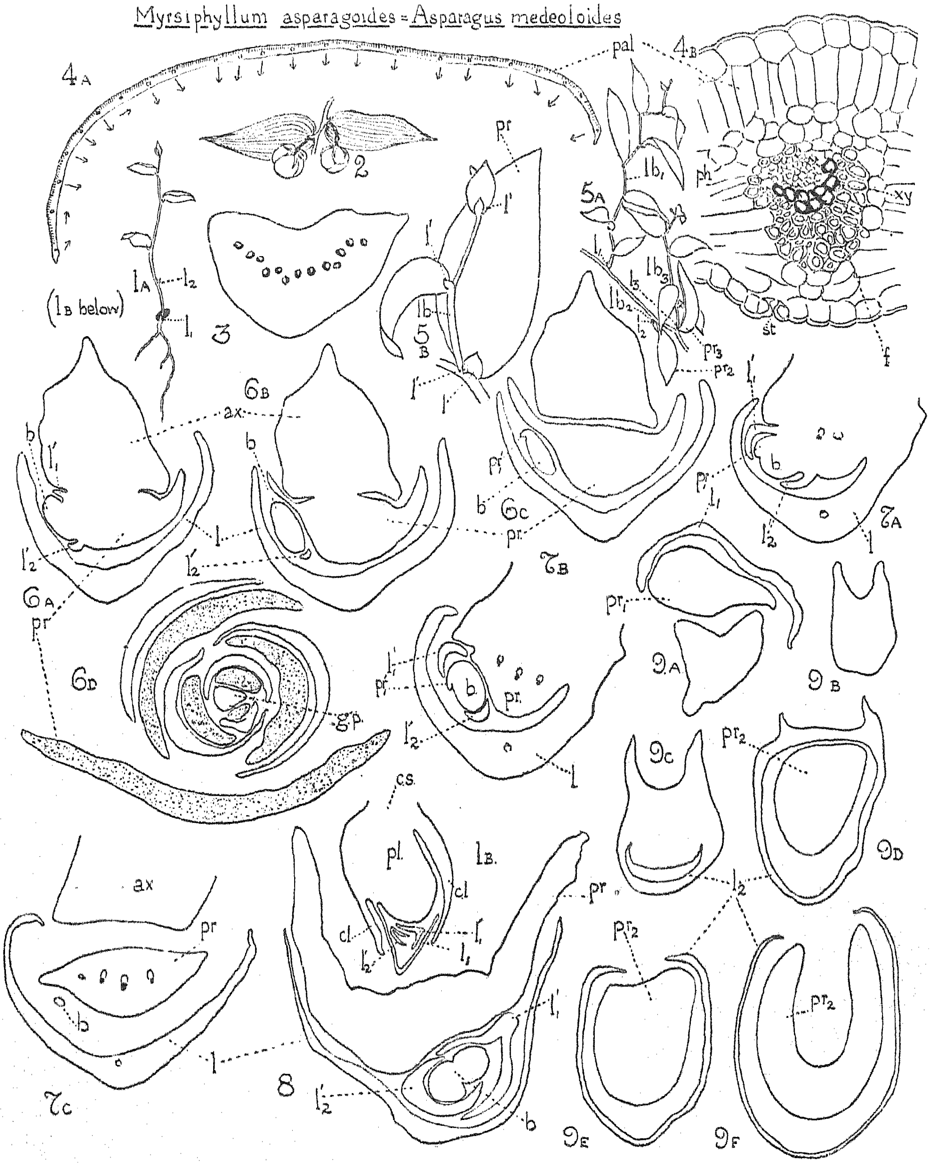
1. INTRODUCTION.

IN a recent paper in the 'Annals of Botany' (5), I have discussed the controversial question of the nature of the 'phylloclades' in the three Liliaceous genera—*Danae*, *Ruscus*, and *Semele*—forming the tribe Rusceae. The interpretation of the assimilating organs, whether of leafy or needle-like form, in the related genus *Asparagus* (including *Myrsiphyllum*) presents a problem of equal, if not greater, difficulty. I propose in the present paper to reconsider this question and, especially, to see whether any light can be thrown upon it by a study of the relations of the parts of the shoot, as revealed in serial sections.



FIGS. 1–9. *Myrsiphyllum asparagoides*, Willd. (*Asparagus medeoloides*, Thunb.). Fig. 1 A, seedling ($\times \frac{1}{2}$); $l._1$, first plumular leaf, a scale almost hidden by cotyledon sheath; $l._2$, second plumular leaf with bud in its axil. Fig. 1 B (near foot of page), transverse section through base of a seedling, such as Fig. 1 A, at level of attachment of cotyledon sucker, *c.s.*, which is cut off ($\times 14$); *c.l.*, flaps of cotyledon sheath; $l._1$, first plumular leaf; $l'._1$ and $l'._2$, first two leaves of bud in axil of $l._1$. Fig. 2, short segment of axis, showing, in the axils of scale-leaves, two phylloclades (under surface) with two fruits, La Mortola, May 28 ($\times \frac{1}{2}$). Fig. 3, transverse section of base of a phylloclade ($\times 23$). Fig. 4 A, transverse section of mature phylloclade ($\times 14$); the axillant leaf would lie towards foot of page; the arrows show direction in which xylem groups of bundles point; *pal.*, palisade parenchyma. Fig. 4 B, small part of transverse section of phylloclade ($\times 193$); *xy.*, xylem; *ph.*, phloem; *f.*, fibres; *pal.*, palisade parenchyma; axillant leaf would lie towards foot of page. Figs. 5 A and B, types of branching. Fig. 5 A, segment of axis bearing three scale-leaves, $l._1$, $l._2$, $l._3$;

I am indebted for material to the Director of the Royal Botanic Gardens, Kew; to the Keeper of the Department of Botany, British Museum (Nat. Hist.); to the Director and to the Superintendent of the Cambridge Botanic Garden; and, especially, to Commendatore Cecil Hanbury, F.L.S., and Mr. J. Benbow, of La Mortola, Ventimiglia.

2. THE 'PHYLLOCLADES' OF *MYRSIPHYLLUM.*

Four species of *Asparagus* from South Africa were included by Baker (7) in the Section *Myrsiphyllum*—*A. medeoloides*, Thunb., *A. undulatus*, Thunb., *A. Krausii*, Baker, and *A. volubilis*, Thunb. These species all have leaf-like phylloclades, and their vegetative morphology is so markedly different from that of *Asparagus* proper, that I think it would be well if *Myrsiphyllum* could be reinstated as a generic name. One of the four species—*Myrsiphyllum asparagoides*, Willd. (*A. medeoloides*, Thunb.), is cultivated in Europe, sometimes under the misnomer 'Smilax', so I have been able to study the plant in the fresh state; in the case of the other three species, I have had to rely upon small quantities of herbarium material.

(i) *Myrsiphyllum asparagoides*, Willd. (*A. medeoloides*, Thunb.).

Myrsiphyllum asparagoides is a graceful climber, whose assimilating organs take the form of flat phylloclades, arising in the axils of scale-leaves. They are typically foliar in appearance (Figs. 1 A, 2, 5 A and B, p. 636); both in their form and in their delicate parallel venation, with no clearly defined midrib, they recall the phylloclades of the Victor's Laurel, *Danae racemosa*, (L.) Mönch. Sections cut near the tips of the very young shoots confirm and emphasize this resemblance, for they show how closely the general relation of leaves and phylloclades in the bud of *Myrsiphyllum* recalls that met with in the Rusceae. Fig. 6 D, for instance, is almost the counterpart of the section of a corresponding bud of *Ruscus aculeatus*, L., such as that shown in 5, Fig. 11 B, p. 236. And this resemblance extends even to such minor

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l.$_1$ bears branch *l.b.*$_1$ in its axil, but no phylloclade; *l*.$_2$ bears a phylloclade, *pr*.$_2$, and an undeveloped bud, *l.b.*$_2$; *l*.$_3$ bears branch, *l.b.*$_3$, with phylloclade, *pr*.$_3$, at its base (× ½). Fig. 5 B, small region of axis, with lateral branch, *l.b.*, similar to *l.b.*$_3$ in Fig. 5 A (with its phylloclade, *pr.*, whose under surface is shown) arising in the axil of leaf *l.*; *l'.* and *l'.*, scale-leaves borne by *l.b.* (slightly enlarged). Figs. 6 A–D, series of sections through a plumule apex (× 47); bud, *b*, in axil of leaf, *l.*, bearing phylloclade, *pr.*, and leaves, *l'*.$_1$, and *l'*.$_2$, and phylloclade, *pr'*.$_1$, in axil of *l'*.$_1$. Figs. 6 A–C are near together. In Fig. 6 D, which is considerably higher, close to the extreme tip of the shoot, the phylloclades are dotted and the axillant leaves left white; *g.p.*, undifferentiated apical cone; *pr.*, upper part of the prophyll shown in Figs. 6 A–C. Figs. 7 A–C, series of transverse sections near shoot apex, showing development of slightly older bud, *b.*, than that in Fig. 6; *l.*, axillant leaf (× 47). Fig. 8, transverse section through an axillant leaf, *l.*, near the apex of a shoot, with phylloclade, *pr.*, and bud, *b.*, in its axil, bearing leaves, *l'*.$_1$ and *l'*.$_2$ (× 47). Figs. 9 A–F, series of transverse sections from below upwards through shoot apex, showing two successive leaves, *l*.$_1$ and *l*.$_2$, and their phylloclades, *pr*.$_1$ and *pr*.$_2$; *pr*.$_2$ is terminal to the shoot (× 47).

peculiarities as the tendency—common among the Rusceae—for the phylloclade sometimes to appear as the terminal member of a shoot; Figs. 9 A–F illustrate a case (the only one I have seen) in which a phylloclade of *Myrsiphyllum* (*pr.*$_{2}$) forms the actual termination of the axis. And when we turn from the general shoot-relations to the vascular scheme of the phylloclade, we find an even more marked agreement between *Myrsiphyllum* and the Rusceae. Transverse sections at the base of the organ reveal an arc of bundles, each with a V or horseshoe of xylem, which in most cases is directed downwards, that is to say, towards the axillant leaf (Fig. 3). Sections of the main part of the phylloclade (Figs. 4 A and B) show this feature even more strikingly, since here the palisade parenchyma lies, as usual, towards the upper surface, but the xylem, which in normal leaves is turned towards the palisade, is, in the phylloclades, directed downwards, i. e. towards the axillant leaf. This unusual orientation is a character which the phylloclades of *Myrsiphyllum* share with those of *Danae*, *Ruscus*, and *Semele* (cp. Figs. 4 A and B of the present paper with 5, Figs. 2 B and C, 4 B, p. 232, 10, 19 D–F, p. 236, 21 D and K, 23 A and B, p. 238, 26, 28, p. 240, and 35, p. 244). A study of the phylloclades of the Rusceae has led me to the conclusion that these organs are the prophylls of reduced shoots, whose axes have no free existence. The phylloclade of *Myrsiphyllum* corresponds so closely to those of the Rusceae that, if my interpretation is correct for *Danae*, *Ruscus*, and *Semele*, it must inevitably be extended to *Myrsiphyllum*. I will not reiterate the argument, so far as it applies equally well to the Rusceae and to *Myrsiphyllum*, since it is treated at length in the paper already cited, but there are certain features, in which *Myrsiphyllum* differs from the Rusceae, which demand attention; these features seem to me, however, to increase rather than diminish the probability of the prophyll interpretation.

Confining our attention for the moment to the non-reproductive shoots, it will be recalled that, in the case of the Rusceae, two kinds of lateral branches occur in the axils of scale-leaves:—

1. Short shoots, in which the rudimentary axis never becomes free from the phylloclade (prophyll).

2. Long shoots, without a differentiated prophyll, but bearing a succession of scale-leaves, which have reduced shoots (belonging to type 1) in their axils.

Both these classes of lateral branch occur in *Myrsiphyllum*, but in addition the following type is found:—

3. Long shoots, bearing a basal phylloclade (prophyll), which is succeeded by scale-leaves, with reduced shoots (belonging to type 1) to their axils.

These three contrasting types are shown in Fig. 5 A, p. 636; the upper phylloclades on branches *l.b.*$_{1}$ and *l.b.*$_{3}$ are borne on reduced lateral axes belonging to type 1; *l.b.*$_{1}$ represents type 2, in which a long shoot, without a basal phylloclade, arises in the axil of a scale-leaf; while *l.b.*$_3$, with its basal phylloclade (prophyll), *pr.*$_3$, is an example of type 3. This third type can be studied more readily in Fig. 5 B, which shows, on a larger scale, a lateral branch, *l.b.*, emerging somewhat asymmetrically between its prophyll (phylloclade), *pr.*, and the axillant leaf, *l.* For the clear understanding of these different shoots, however, naked-eye observations are insufficient; serial sections are required in order to determine the precise relations of the parts. The form of branch which we have called type 2 is seen in section, at a very young stage, in Fig. 1 B (near foot of page); the section traversed the base of a plumule, such as that of the seedling drawn in Fig. 1 A; it is characteristic of the first plumular leaf, *l.*$_1$, that it makes its appearance very low down, between the two flaps of the cotyledonary sheath, *c.l.*[1] This leaf, *l.*$_1$, has in its axil a bud, which has produced two lateral scale-leaves, *l'.*$_1$ and *l'.*$_2$; it will be noticed that there is no prophyll facing the axillant leaf. Figs. 6 A–C, on the other hand, show a series of sections through the origin of a branch belonging to type 3, in which there is a basal phylloclade, *pr.* These sections are slightly bewildering at first glance, but they explain themselves when one realizes that the unusual appearance of the lateral branch is mainly due to the fact that its terminal bud, *b.*, is pushed to one side by the excessive development of its prophyll (phylloclade), *pr.*, which itself remains attached dorsally to the parent axis, after the apex, *b.*, has become free (Fig. 6 B; see also Fig. 7 B). The fact that, instead of embracing the daughter axis to which it belongs, the margins of the phylloclade bend, as it were, backwards round the parent axis, so that it lies more or less parallel to the axillant leaf, is also due, like the deflexion of the apex, to the exaggerated size of the phylloclade, and the confined space in which it has to develop. In these sections, two further leaves, *l.'*$_1$ and *l.'*$_2$, and a third rudiment, *pr'*$_1$., which is probably the phylloclade in the axil of *l'.*$_1$, follow the phylloclade (prophyll); they are distichously arranged, and, but for the displacement of the lateral bud, they would lie at right angles to the prophyll.[2]

Figs. 7 A–C are from a similar but slightly older bud. Fig. 8 represents another branch at a still more advanced stage; in this case the lateral shoot, *b.*, is less deflected from the median line than in the other examples illustrated.

I regret that I have not been able to study reproductive shoots, but the appearance of the infructescences (Fig. 2) indicates that the mode of branching is allied to that described, for the vegetative shoots, as type 3.

An inspection of Figs. 6, 7, and 8 suggests that, in *Myrsiphyllum asparagoides*, we have a case which makes the anomalous morphology of the

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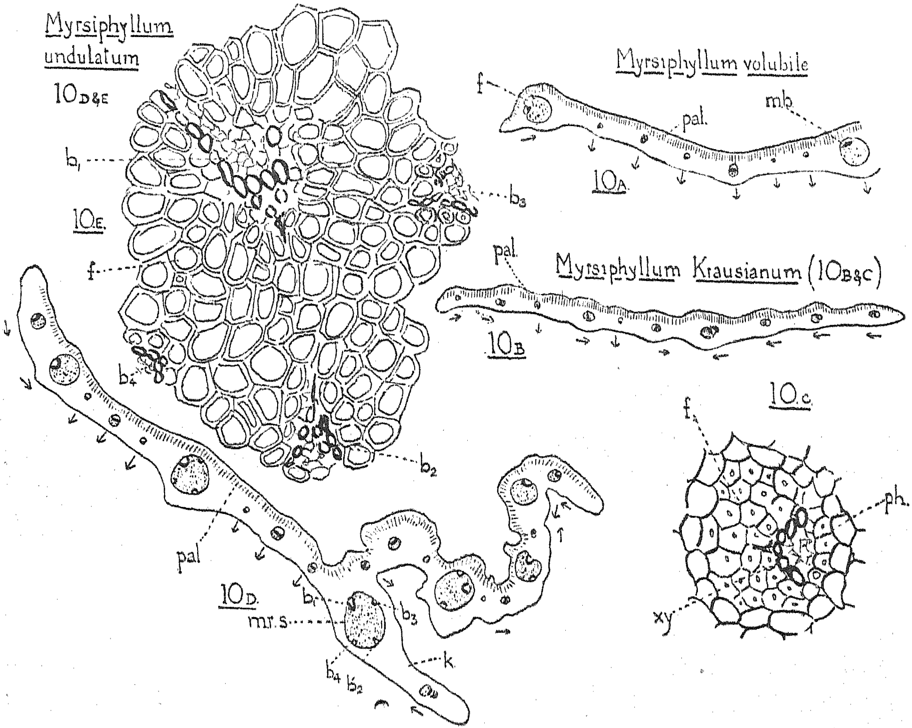
[1] For an account of the same feature in *Asparagus* proper, see 14.

[2] *l'.*$_1$ and *l'.*$_2$ arise almost at the same level; indeed the leaf which I have called *l'.*$_2$ may sometimes begin to make its appearance a little before *l'.*$_1$.

Rusceae easier to understand. In both instances the key to the problem lies in the recognition of the 'phylloclade' as the overgrown prophyll of a lateral shoot, but whereas, in the Rusceae, this lateral shoot remains abortive, or, if it continues to grow, never becomes free from the prophyll, in *M. asparagoides* it sometimes grows on, and forms a normal elongated axis, bearing a succession of leaves. *Myrsiphyllum* thus offers a link between the Rusceae and other Monocotyledons, whose vegetative system follows more ordinary lines.



FIGS. 10 A–E, phylloclades of *Myrsiphyllum*; throughout, *f.*, fibres; *xy.*, xylem; *ph.*, phloem; *pal.*, palisade parenchyma; arrows indicate direction of xylem. Fig. 10 A, *M. volubile* (*Asparagus volubilis*, Thunb.), part of transverse section of phylloclade (× 23); *m.b.*, median bundle. Figs. 10 B and C, *M. Krausianum*, Kunth (*A. Krausii*, Baker); Fig. 10 B, transverse section of phylloclade (× 23); Fig. 10 C, midrib strand (× 318). Figs. 10 D and E, *M. undulatum*, Schlecht. (*A. undulatus*, Thunb.); Fig. 10 D, transverse section of phylloclade (× 23); *k.*, keel; *m.r.s.*, midrib strand consisting of four bundles, $b._1$, $b._2$, $b._3$, and $b._4$, embedded in fibres; Fig. 10 E, midrib strand from Fig. 10 D (× 193).

(ii) *Myrsiphyllum volubile* (*Asparagus volubilis*, Thunb.), *M. Krausianum* Kunth (*A. Krausii*, Baker), and *M. undulatum*, Schlecht. (*A. undulatus*, Thunb.).

It now becomes necessary to compare the phylloclade of *Myrsiphyllum asparagoides* with that of the remaining members of the Section, in order to see if the interpretation advanced for *M. asparagoides* is applicable to the other species. In each of the three species in question, the phylloclade resembles that of *M. asparagoides* in being leaf-like in appearance, but the anatomy is somewhat different, and the development of fibres is much greater.

Of *Asparagus volubilis*, which I should prefer to call *Myrsiphyllum volubile*, I have only been able to examine two detached phylloclades (Fig. 10 A, p. 640). But these agree with *M. asparagoides* in having the xylem of the majority of their bundles directed away from the palisade tissue, and therefore, presumably, towards the axillant leaf. The marginal bundles, however, are placed horizontally, with their xylems pointing in the direction of the midrib.

In the case of *Myrsiphyllum Krausianum*, again, I have only had two phylloclades, without the axis which bore them, so that the orientation could not be determined with certainty. But two or three of the smallest bundles are placed so that their xylems are turned away from the palisade tissue, as in the two preceding species, while the majority lie with their plane of symmetry parallel to the surface of the phylloclade; this is the case even in the midrib (Figs. 10 B and C). I am inclined to think that this peculiar orientation, which I have never seen completely paralleled in any other foliar organ, is merely the extreme development of a tendency which is already, to some degree, operative in the other species. In *M. asparagoides*, the great majority of the bundles, like most foliar strands, are symmetrical about a plane at right angles to the surface of the phylloclade, but a few are placed horizontally (Fig. 4 A, p. 636). In *M. volubile*, so far as my observations go, a single bundle at each margin has this horizontal placing (Fig. 10 A), while in *M. undulatum* this character is shared by two bundles at each margin (Fig. 10 D). If further bundles were to become involved, until a stage were reached in which all the principal strands were orientated perpendicularly to the usual plane, the type of structure met with in *M. Krausianum* would result. Occasional horizontally-placed bundles are not unknown in organs about whose foliar nature there is no dispute. The marginal bundles of the phylloclades of *Oxalis bupleurifolia*, A. St.-Hil. (1, Fig. 3 B, p. 474), and of the leaves of *Ophiopogon japonicum*, Ker-Gawl. (2, Fig. 38, p. 464), and of *Allium Dioscoridis*, Sibth. et Sm. (2, Fig. 30 B, p. 459), share the same peculiarity. But the point which concerns us chiefly now is that this type of orientation may be observed, though rarely, in the Rusceae; for individual bundles in the phylloclades of *Danae racemosa* and *Ruscus aculeatus* sometimes lie horizontally.

In the case of the third species—*Myrsiphyllum undulatum*, Schlecht.—I was fortunate in having a piece of axis with several phylloclades attached, so that I could determine the orientation; in Fig. 10 D, which shows the transverse section of the phylloclade, the axillant leaf would lie towards the foot of the page, while the palisade is on the upper surface, which is turned away from the axillant leaf. In this respect the phylloclade resembles that of *M. asparagoides*; its form is, however, somewhat different, as there is a median keel, *k.*, which is not found in any of the other species. The minor bundles, as in *M. asparagoides* and *M. volubile*, have their xylems directed away from the palisade parenchyma, i. e. towards the axillant leaf, while the two marginal bundles on each side have their xylems directed towards the midrib, like the marginal strands of *M. Krausianum.* There is, however, one noticeable difference between *M. undulatum* and the three other species—namely, that the midrib and the principal lateral bundles are supplied by steles including several bundles radially arranged, instead of single bundles. Fig. 10 E shows the detailed structure of the midrib strand, which consists of four bundles embedded in fibres. But this peculiar anatomy does not invalidate the prophyll interpretation, since we find an exactly comparable type of structure in the phylloclades of *Ruscus Hypoglossum*, L., *R. Hypophyllum*, L., and *Semele androgyna*, (L.) Kunth, in which even the non-flowering phylloclades have radial bundle-groups in their principal veins (compare Figs. 10 D and E with 5, Fig. 23 B, p. 238, Figs. 26 B and 27 E, p. 240, and Fig. 45 B, p. 246).

A minor anatomical feature, common to the phylloclades of all four species of *Myrsiphyllum*, is the occurrence of a relatively enormous development of fibres on the side of the bundle remote from the phloem; this arrangement is shown in detail for *M. asparagoides* in Fig. 4 B, p. 636, and for *M. Krausianum* in Fig. 10 C, p. 640. This is the reverse of the usual scheme, in which the fibres lie outside the phloem, but it is not without parallel in the leaves of Monocotyledons; I have observed it in *Xyris brevifolia*, Mich. (4, p. 83 and Pl. II, Fig. 18). It is, however, an extremely rare condition, and its appearance in these four species belonging to the *Myrsiphyllum* group confirms the view that they deserve to be segregated from *Asparagus* and given generic rank.

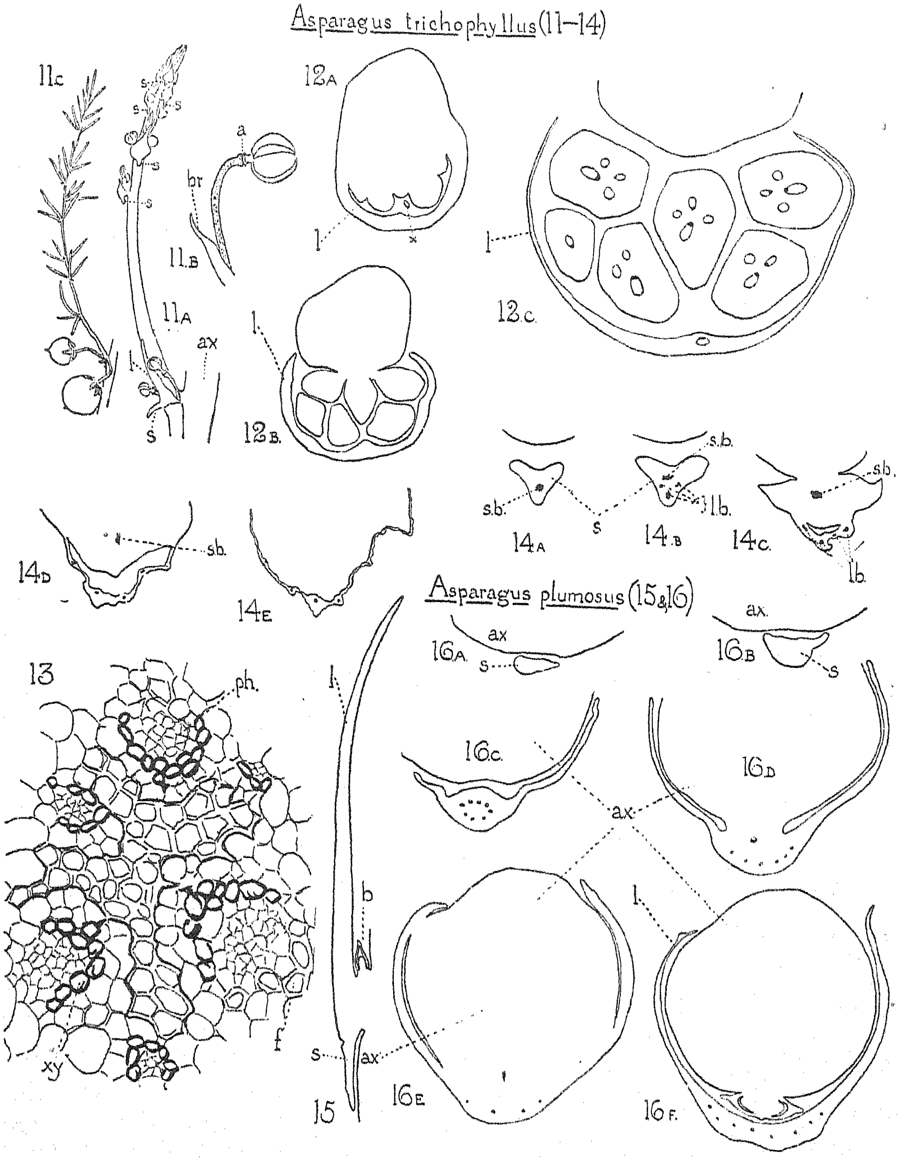
We may, I think, conclude, from this brief account of the phylloclades of *M. volubile*, *M. Krausianum*, and *M. undulatum*, that the facts of their structure strengthen the case for the morphological identification of the phylloclades of *Myrsiphyllum* with the phylloclades (prophylls) of the Rusceae.

(iii) *Historical.*

I have begun by putting forward my own view of the phylloclade of *Myrsiphyllum*, instead of first treating the subject historically, because it seems to me that the morphology of the genus has never, hitherto, been considered *on its own merits*; each author who has dealt with the question has assumed that the relationship between *Myrsiphyllum* and *Asparagus* is so close that any theory which is held about the nature of the 'needles' of *Asparagus* applies automatically to the phylloclades of *Myrsiphyllum*, or rather to those of *M. asparagoides*, Willd., which is the only species which seems ever to have come under discussion. Kunth (17), so far as I know, was the earliest writer to term the assimilating organs, both of *Myrsiphyllum* and *Asparagus*, sterile flower-stalks, but without giving reasons for his opinion. Čelakovský (11), again, assumes the homology of the phylloclades of *Myrsiphyllum* and the needles of *Asparagus*, but brings forward no other evidence (at least in his German abstract) for the view that they are axial. Goebel (15 and 16), also, while admitting that the phylloclades of *Myrsiphyllum asparagoides* 'have entirely the structure of leaves', yet holds, by analogy with the needles of *Asparagus*, that they must, of necessity, be axial. Velenovský (24), again, although, in opposition to Goebel, he upholds the view that the phylloclades of *Danae* are foliar, nevertheless concludes that, despite their great apparent similarity, the phylloclades of *Danae* and *Myrsiphyllum* cannot be homologous organs, because those of *Myrsiphyllum* are axial like the needles of *Asparagus*. Indeed, the principal supporters of the theory that the phylloclades of *Myrsiphyllum* are stem structures agree in never having brought forward any argument in support of this opinion, which seems to be an unwarranted deduction from conclusions arrived at from the study of *Asparagus* (*sensu stricto*). I hope, however, to show in a later section of this paper that the needles of *Asparagus* differ so widely from the 'phylloclades' of *Myrsiphyllum*, both in their relation to the rest of the shoot and in their structure, that it is highly improbable that the same morphological interpretation can be made to serve for both organs.

3. THE SPINOUS SCALE-LEAVES OF *ASPARAGUS*.

The scale-leaves of many species of *Asparagus* are characterized by a downward basal outgrowth in the median plane, which may become hard and spinous at maturity. These spines (*s.*) can be seen in Fig. 11 A, p. 644, which represents a young branch of *A. trichophyllus*, Bunge. Fig. 15 shows a longitudinal section through a single young scale-leaf of *Asparagus plumosus*, Baker, in which the tail or spine is well developed, while Figs. 16 A–F represent a series of transverse sections passing upwards from below through a similar scale-leaf. Figs. 14 A–E are, however, of greater interest, since it is possible to make out from them the rather curious vascular scheme of the organ. Fig. 14 D shows a bundle, *s.b.*, passing horizontally from the axis towards the leaf; it turns directly down into the spine (Fig. 14 A). The upper scale-like portion of the leaf is supplied by branches (*l.b.*) arising from the spine bundle on the side remote from the axis. In *Asparagus officinalis*, L. (Figs. 19 A–E, p. 648), the same scheme is followed. Here the bundle, *s.b.*, passes down into the spine, forming, near the tip, a vascular complex (Fig. 19 A). From this bundle three strands (*l.b.*) are given off on the dorsal



FIGS. 11–16. Figs. 11–14, *Asparagus trichophyllus*, Bunge. Fig. 11 A, lateral branch with flower-buds borne on main aerial axis, *ax.* (about nat. size); *l.*, axillant leaf with spine, *s.* Fig. 11 B, flower-bud enlarged to show articulation, *a*, near top of pedicel, and basal bract, *br.* The dotting indicates change of colour from green to brownish below articulation. Fig. 11 C, lateral branch with a pair of fruits at the base (× ½). Figs. 12 A and B, two sections from series from below upwards through young group of six needles in axil of leaf, *l.*; the process marked × is possibly a seventh needle (× 23). Fig. 12 C, a third section, a little higher, on a larger scale to show vascular tissue (× 47). Fig. 13, central cylinder from a lateral member of a group of three mature needles (× 318) showing six bundles; *xy.*, xylem; *ph.*, phloem; *f.*, fibres. Figs. 14 A–E, series of sections from below upwards through an axillant scale-leaf (× 14); Figs. 14 A and B show the free spine, *s.*; Fig. 14 C, attachment to axis; *s.b.*, vascular bundle of spine; *l.b.*, branches given off from *s.b.* to supply leafy

side and enter the upper part of the leaf. Exactly the same process takes place in *Asparagus scaber*, Brign. (Figs. 38 A–F, p. 655), though here the spine of the scale-leaf looks externally rather different, since it bears irregular emergences (Fig. 37). It is probable that the vascular scheme of the scale-leaf of *Asparagus plumosus* (Figs. 16 A–F, p. 644) follows the same lines, but in the leaves which I cut the differentiation of the bundle-tissue in the spine was incomplete.

Clos (12), in whose paper references to earlier work will be found, treated the spines of *Asparagus*, not as belonging to the leaf, but as processes from the 'coussinet'; Goebel (15), on the other hand, interprets the spines as outgrowths from the under side of the leaf. In view, however, of the anatomical structure just described, it is hard to see how either of these opinions can be maintained. For the main fact which emerges from the study of the vascular system is that the spine is an integral and important member of the leaf, since it receives its vascular supply direct from the axis, while the upper scale-like part of the leaf is supplied—secondarily—from the vascular tissue of the spine. I wish here to support an interpretation—totally different from that of Clos or Goebel—first indicated in 1920 (9) in a preliminary note by Professor Luigi Buscalioni of Catania, who very kindly communicated his idea to me more fully in a letter dated May 20, 1921. According to this view, the spinous leaf of *Asparagus* is a phyllode with no true lamina, in which the basal leaf-sheath is also absent, but the petiole is present in the form of a reflexed spine, while the upper scale-like part of the leaf is a ligular sheath. It may be difficult, at first glance, to visualize the relation borne by these curious leaves to others of a more normal type, but an analogy can be found in certain seedlings, in which the petiolar limb of the cotyledon is directed obliquely downwards. The seed-leaf of *Tigridia* (Iridaceae), for instance, bears a close resemblance to the spinous scale-leaf of *Asparagus*, for it has no basal sheath, but consists entirely of a sheathing ligule, continued basally into the cotyledon sucker, which is directed obliquely downwards (21, Fig. 15, p. 168). I believe that the spine of the *Asparagus* scale is equivalent to the sucking part of the cotyledon in *Tigridia*, and that both these organs are of petiolar nature.

4. THE 'NEEDLES' OF *ASPARAGUS*.

(i) *Historical.*

The vegetative shoots of *Asparagus* bear scale-leaves in whose axils arise groups of assimilating organs, which are generally slender and acicular. Throughout the present paper, I am using the term 'needles' for these

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part of scale. Figs. 15 and 16, *Asparagus plumosus*, Baker. Fig. 15, longitudinal section of leaf, *l.*, with spine, *s.*, and axillary bud, *b.*, from near apex of young axis, *ax.*, February (× 14). Figs. 16 A–F, series of transverse sections from below upwards through a leaf and bud, February; lettering as in Fig. 15 (× 14).

organs—whatever their exact form—because it is a non-committal term which carries no morphological implication.

De Candolle in 1827 (10) interpreted the needles of *Asparagus* as reduced leaves borne on short shoots like those of *Pinus*, and a few other writers, notably Buscalioni (9), have held some version of the foliar view (see also 23). But the great majority of observers have regarded the needles as axial organs, probably equivalent to flower pedicels. The first expression of this view is, I believe, that of Link in 1798 (18); in the early nineteenth century, he was followed by Bravais (8), Döll (13), Wydler (25), and Kunth (17), and this interpretation has been almost universally accepted by modern writers; it is supported, for instance, by Čelakovský (11), Reinke (20), Velenovský (24), and Goebel (16). But though this view has been so very widely adopted, it has seemed to me, on studying the literature, that it has been accepted rather as an article of faith than as a conclusion from the balance of probabilities; singularly little evidence appears ever to have been adduced, either for or against it. I have therefore made a re-examination of the vegetative organs of the *Asparagus* shoot, in order to try and determine which of the two opposed interpretations—the foliar or the axial—receives most support from the actual facts.

(ii) *The Relation of Vegetative Axes, Pedicels, and 'Needles'.*

In certain species of *Asparagus*, the flowers are borne on special lateral axes, which produce no needles, e. g. *A. Sprengeri*, Reg. (Fig. 36 A, p. 651). But, in the more familiar cases, vegetative branches, flowers, and needles are grouped in a fashion which may almost be described as indiscriminate. Fig. 11 A, p. 644, shows a young shoot of *A. trichophyllus*, Bunge, bearing flower buds, while Fig. 11 C represents one of the laterals of such a shoot at the fruiting stage. Here the node bore one needle, one vegetative branch, and two pedicels; but this scheme is only a single example from a large number of possible groupings. I examined a clump of this species, in order to see if there were any regularity in the relation, at the nodes, of vegetative shoots, flowers, and needles, but I could discover none. I found that the nodes in this particular clump could be assigned, according to the organs which they bore, to as many as seventeen classes, which may be summarized as follows:—nodes bearing 3, 4, 5, 6, or 7 needles alone; nodes bearing 4, 5, 6, 7, 8, or 9 needles and 1 vegetative branch; nodes bearing 2, 3, 4, or 5 needles, 1 vegetative branch, and 1 pedicel; nodes bearing 1 or 3 needles, 1 vegetative branch, and 2 pedicels (e. g. Fig. 11 C, p. 644). The inspection of such a clump certainly leaves on one's mind the impression that it would not be surprising if these vegetative shoots, pedicels, and needles were morphologically equivalent. Nevertheless, the fact that these organs can replace one another with such facility does not amount to a *proof* of the axial theory of the needles; evidence of a more stringent character is needed before its validity can be regarded as established.

It is usual in *Asparagus* for the needles to be grouped in smaller or larger fascicles, with a tendency to symmetry about the median plane. Fig. 29, p. 651, shows a small group of needles belonging to *A. Sprengeri*, Reg. They arise in the axil of a reduced leaf, *l.*, and are associated basally with small scale-like structures, which we will consider a little later (p. 650). The needles marked W and X are the first to become free; at a slightly higher level, Y and Z are detached, while the central needle, V, is the last to become independent. Indications of a corresponding order of origin are seen in sections of very young needle-fascicles, such as Figs. 30 G and 31 F, p. 651. The order of detachment of the needles in this and other species is often puzzling to follow, because the separation is sometimes almost instantaneous and the needles themselves may be very numerous. It is difficult, for instance, to draw exact conclusions about the order in such cases as *A. plumosus*, Baker (Figs. 18 A and B, p. 648), *A. retrofractus* (Figs. 39 A–C, p. 655), and *A. sellowiana* (Figs. 46 A and B, p. 657). However, when the fascicles are five-needled, there seems to be a general tendency for the needles W and X to be the first to become free, and for their tips not to reach so high a level as those of Y and Z, which are again overtopped by V. Fig. 28, p. 649, gives an idea of the general scheme in the case of *Asparagus officinalis*.

We have so far considered only needle-groups, pure and simple, but there are also more complex cases, in which flowers and vegetative branches are associated with needles in the axil of the same leaf. In Figs. 19 A–N, p. 648, we can trace, in a series of sections taken from below upwards, the history of an axillant leaf of *Asparagus officinalis* and the structures which it subtends; these consist of a vegetative shoot, A, bearing, basally and laterally, two needles, *n.*, and two flowers, *f.* Fig. 20 is a similar case, but the flowers themselves are seen in greater detail. The central vegetative shoot (A in Figs. 19 F, G, H) is traced further, though not to its apex, in Figs. 19 I–N; it bears axillant leaves and needles. We may, I think, admit that these serial sections lend colour to the idea of the equivalence of needles, flowers, and vegetative shoots; it seems not unnatural to suppose that the five organs (2 needles, 2 pedicels, and 1 vegetative shoot) within the axillant leaf, *l.*, in Figs. 19 F and G, correspond to the five organs (5 needles) within the axillant leaf, *l'.*, in Figs. 19 L, M, N. Wydler (25), however, whose views are expressed in a diagram, which has been frequently used by later writers,[1] goes further than this, and definitely interprets the needle- and flower-group of *Asparagus officinalis* as a partially sterilized inflorescence—a double cyme, comparable with that of *Lamium* or *Galeobdolon*. This view is also adopted

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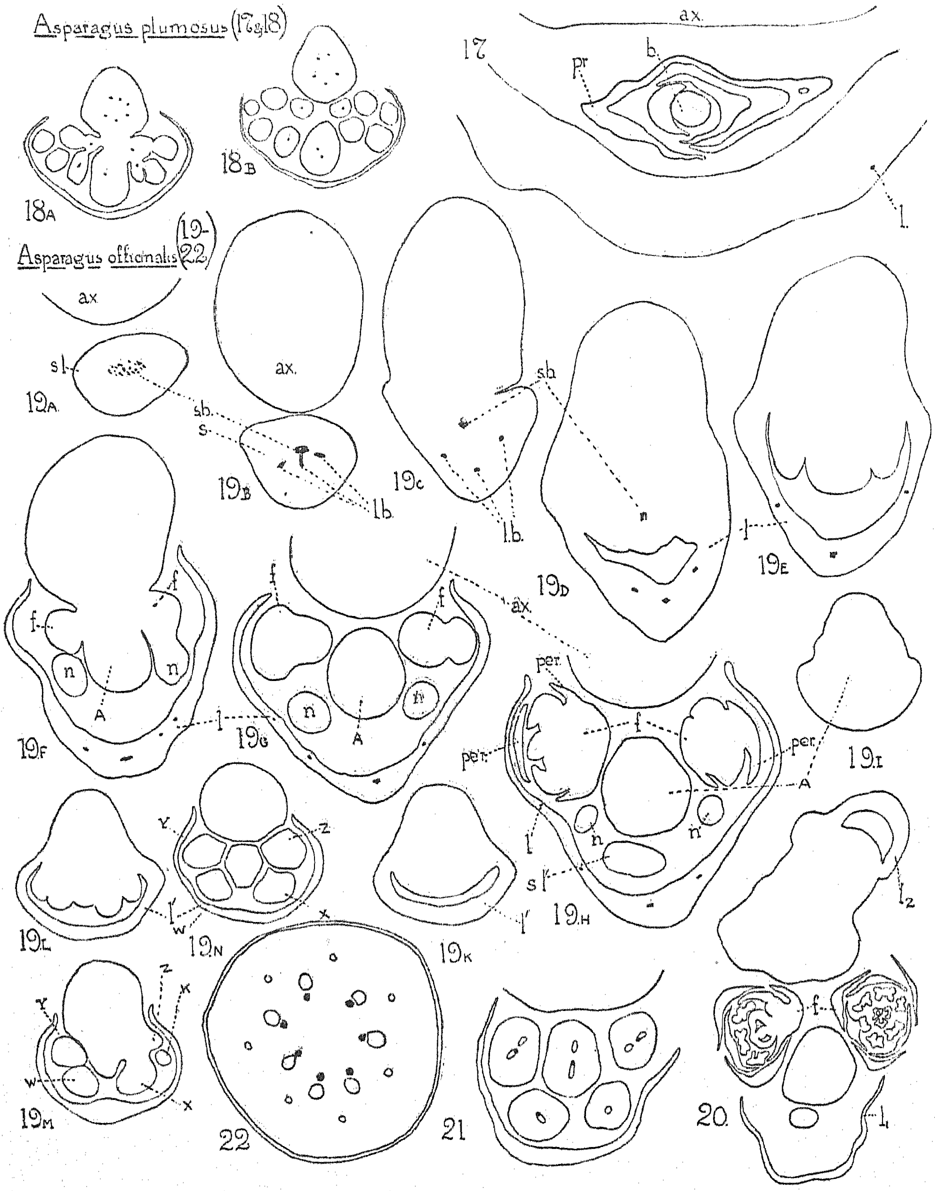
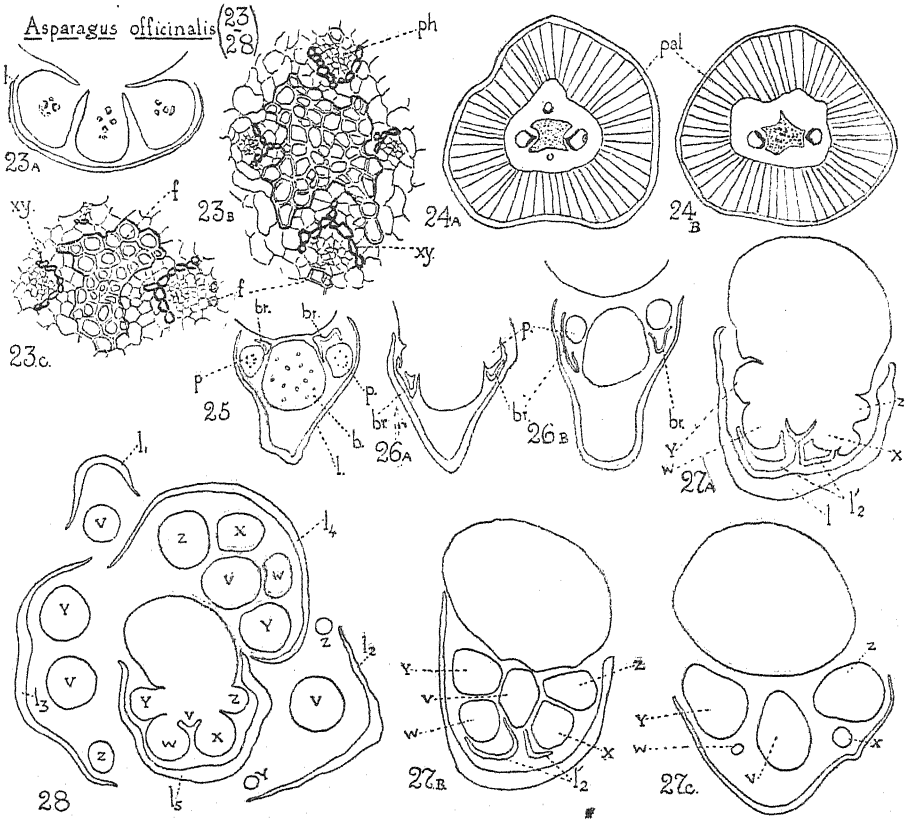
[1] See, for example, Engler and Prantl, Pflanzenfamilien, II, v. 1888, p. 77, Fig. 56.



FIGS. 17–22. Figs. 17 and 18, *Asparagus plumosus*, Baker; Fig. 17, transverse section of part of axillant leaf, *l.*, and axis, *ax.*, to show bud, *b.*, with prophyll, *pr.*, February (× 47). Figs. 18 A and B, two sections of a fascicle of needles from an older shoot. Figs. 19–22, *A. officinalis*, L.; Figs. 19 A–N, series of transverse sections from below upwards through a young shoot (× 47); *ax.*, axis; *s.l.* and *s.l'.*, tails of axillant leaves, *l.* and *l'.*; *s.b.*, vascular bundle of *s.l.*; *l.b.*, bundles given off from *s.b.* to supply upper part of leaf *l.*; *n.*, *n.*, W, X, Y, Z, needles; *f.*, flowers; *per.*, perianth members; no vascular tissue represented except the xylem of leaf *l.* From Fig. 19 I onwards, development of central axis, A, alone followed. Fig. 20, section through another shoot, for comparison with Fig. 19 H, on a smaller scale, but showing flowers, *f.*, more completely formed; l_1 and l_2, axillant leaves (× 14); see Fig. 25, p. 649, for bracts at the base of these pedicels. Fig. 21, group of young needles in axil of leaf *l.*, to show vascular system (× 47). Fig. 22, transverse hand section of mature pedicel (× 23).

by Payer (19). Wydler represents the two flowers as the first lateral organs to be formed, while he regards the first two needles as branching from the base of the pedicels, and the succeeding needles as branching from one another in regular succession on alternate sides. But, according to my observations, the two needles, *n.* and *n.* in Fig. 19 F, are the earliest organs to be detached, becoming independent before the flowers, *f.* and *f.*, have freed themselves either from the parent- or the daughter-axis: and I have



FIGS. 23–8, *Asparagus officinalis*, L. Fig. 23 A, transverse section of group of three needles in axil of shrivelled leaf, *l.*, showing their attachment to one another and to the axis (×47). Figs. 23 B and C, central cylinder of middle needle (B) and one of the lateral needles (C) of Fig. 23 A, at a higher level (×318); *xy.*, xylem; *f.*, fibres; *ph.*, phloem. Figs. 24 A and B, hand sections, lower (A) and higher (B), in a single needle (×77); *pal.*, palisade parenchyma. Fig. 25, bud axis, *b.*, bearing two lateral bracts, *br.*, and flower pedicels, *p.*, in axil of leaf, *l.* (×14); the flowers borne on these pedicels are shown in Fig. 20, p. 648. Figs. 26 A and B, two transverse sections showing another pair of pedicels from the same shoot as Fig. 25, but in which the bracts are differently placed (×14). Figs. 27 A–C, transverse sections from series through group of needles in axil of leaf *l.*, to show scale-leaves, $l'._2$, associated with needles W and X, which are not pedicels, but terminate upwards without further differentiation, as shown in Fig. 27 C, cut just below their tips (×47). Fig. 28, transverse section of young shoot showing cycle of leaves, $l._1$–$l._5$, each with needles in its axil; in passing upwards, the needles W and X of each group first disappear; then Y and Z; and, finally, V is left alone (×47).

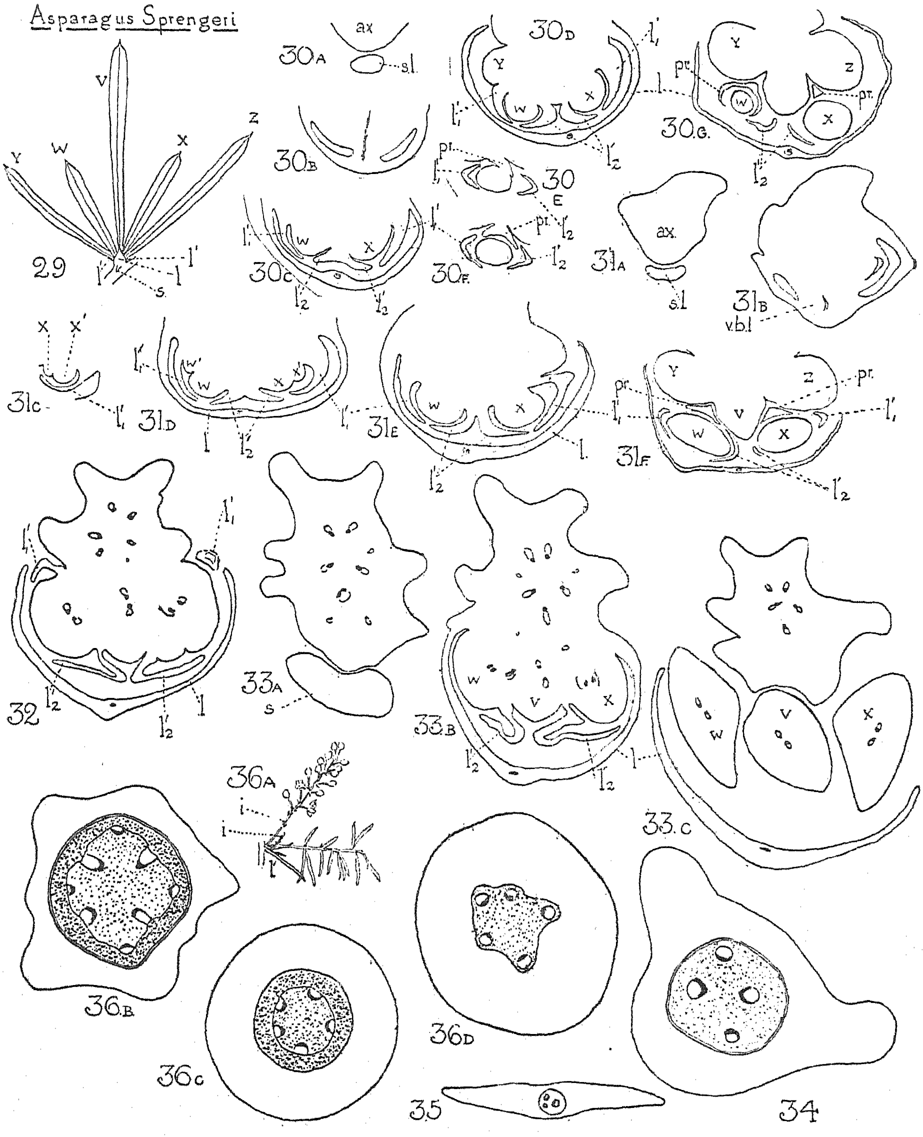
been unable, either in such a case as this, or in fascicles consisting of needles alone, to determine any *constant* order of origin for the needles after the first two. I have thus been unable to find any reality corresponding to Wydler's diagram, and I am forced to the conclusion that this diagram merely represents the theoretic idea which he had formed. My failure to confirm Wydler's description in no way, however, affects the probability of the axial theory of the needles; perhaps it rather removes a difficulty, for it would be curious if these needles, whose relation with the flower-pedicels seems an intimate one, would achieve a cymose grouping, when, so far as I can learn, the inflorescences of *Asparagus* proper are always of the racemose type.

(iii) *The Scale-leaves associated with the Pedicels.*

In considering the group of organs arising within the axillant scale-leaves of *Asparagus*, we have omitted all consideration of the small membranous scale-like structures, which are sometimes, though not always, present. A basal bract may be associated with the flower-pedicel (*br.*, Fig. 11 B, p. 644) and it is possible that the articulation, *a.*, which occurs part way up the pedicel, may represent the last trace of one or more bracteoles. The bracteole explanation of the articulation is confirmed by the difference in colour above and below it, indicated in Fig. 11 B; the surface of the distal part of the pedicel would be perianth-leaf-skin, while that of the basal region would be bracteole-leaf-skin (22). The bracts associated with the pedicels are seen in serial sections of shoots of *A. officinalis* in Figs. 25 and 26, p. 649; it will be realized, on comparing the bracts, *br.*, in Figs. 25 and 26 A and B, that their position is variable; it is doubtful whether one should consider them to be bracts or bracteoles. In another case I followed serial sections through a pair of flowers without finding any trace of a bract in connexion with either. This case is illustrated in Figs. 19 F–H, p. 648; the structures marked *per.*, which might perhaps be taken for bracts, are, in reality, perianth members.

(iv) *The Scale-leaves associated with the 'Needles'.*

That the bracts, discussed in the preceding paragraph, occur in association with the pedicels of *Asparagus* is a matter of common knowledge (6), but nothing comparable, so far as I know, has hitherto been observed in connexion with the needles; Goebel, in fact, definitely states that these organs are quite leafless (16). I was therefore surprised to find, among my serial sections of non-reproductive shoots of *A. officinalis*, one 5-needled fascicle, in which two of the needles (W and X, Figs. 27 A and B, p. 649) each bore a small scale-leaf ($l'._2$), resembling the bract of a pedicel. It will be recognized, from Fig. 27 B, that W and X are undoubtedly needles and not pedicels, for they terminate in pointed apices, quite unlike the rudimentary flowers seen in other sections. This was the only example of the kind



FIGS. 29–36, *Asparagus Sprengeri*, Reg. Fig. 29, group of five needles (V, W, X, Y, Z) in axil of leaf, *l.*, with spine, *s*; *l'*., small scales at base of needles (nat. size). Figs. 30 A–G, series of transverse sections through one leaf, *l.*, with its spine, *s.l.*, and the five needles, V, W, X, Y, Z, in its axil, to show leaves *l'*.$_1$, *l'*.$_2$, *pr.*, associated with the needles W and X (× 47). Figs. 31 A–F, series similar to that in Figs. 30 A–G, but showing lobing in case of W and X, which gives rise to the rudimentary needles W' and X' (× 47). Fig. 32, transverse section of base of three needles, somewhat older than that shown in Figs. 30 and 31 (× 47); in this case *l'*.$_1$ and *l'*.$_2$ are developed on each side, but not *pr.* Figs. 33 A–C, transverse sections from series through axillant leaf, *l.*, with spine, *s.*, and group of three needles in its axil (× 47); in this case only the leaves *l'*.$_2$ are developed. Fig. 34, transverse section near base of a mature needle showing beginning of winged form (× 77). Fig. 35, transverse hand section of mature needle to show winged form (× 14). Figs. 36 A–C, inflorescence. Fig. 36 A, node with two inflorescences, *i.* and *i.* (one cut off short), associated with needles and a vegetative branch, in the axil of a scale-leaf, *l.* (× ½). Fig. 36 B, transverse section of main axis of inflorescence (× 77). Figs. 36 C and D, transverse sections of pedicel (C) below and (D) above the articula-

which I met with in *A. officinalis*, but in *A. Sprengeri* the association of the needles with scale-leaves is a much more constant and striking feature. These scales can sometimes be recognized, even with the naked eye, as small scarious structures at the bases of some of the mature needles (Fig. 29, *l.'*, p. 651). Fig. 33 B, p. 651, shows a section of a 3-needled group, in which there is a development of two leaves ($l'_{\cdot 2}$ and $l'_{\cdot 2}$), corresponding in position with the two leaves observed in *Asparagus officinalis* (Figs. 27 A and B, p. 649). In another 3-needled group (Fig. 32, p. 651) I found four leaves ($l'_{\cdot 1}$, $l'_{\cdot 1}$, $l'_{\cdot 2}$, $l'_{\cdot 2}$). The fullest leaf development is, however, found in such 5-needled groups as those drawn in Figs. 30 and 31, in which six leaves are associated with the needles. The delicate non-vascular structure of these leaves, and the fact that they originate at the base of the group of needles, at a level at which these have not completely freed themselves from the axis, or from one another, makes it difficult at first to understand the relation of leaves and needles, but I have come to the conclusion that this relation is a definite and regular one. It will be seen that the leaves are associated exclusively with the needles W and X; $l'_{\cdot 1}$ and $l'_{\cdot 2}$ form an opposite pair more or less at right angles to *l.*, the axillant leaf of the whole group, while *pr.* is parallel to the axillant leaf, which it faces. Now there seems to me no doubt that $l'_{\cdot 1}$, $l'_{\cdot 2}$, and *pr.* are to be interpreted as leaves *borne upon* the needles W and X, which are hence necessarily axial organs. The only alternative is to suppose that these leaves are really borne on the main axis, or on an axial base common to the group of needles; it is possible that such a section as Fig. 31 E, in which the leaves are only partially free from the axis, might be pointed to in support of this view. But I do not think that this contention can possibly be maintained, in view of the actual position of the leaves, especially when we realize that, in other cases, leaves borne on what are undoubtedly lateral axes may yet be attached at the base to the main axis. Figs. 45 A and B, p. 657, for instance, represent the origin of one lateral bud ($b_{\cdot 1}$) and two secondary lateral buds ($b_{\cdot 2}$) in the case of a turion of *Asparagus sellowiana*. The buds, $b_{\cdot 2}$ and $b_{\cdot 2}$, arise very early, before their parent axis, $b_{\cdot 1}$, has become free from the main axis, *ax.*, and just at their bases (Fig. 45 B) the leaves of the third order, which, in Fig. 45 A, I have labelled $l_{\cdot 3}$, show the same lack of freedom from the parent axis, $b_{\cdot 1}$, as do the leaves $l'_{\cdot 1}$ and $l'_{\cdot 2}$ in Figs. 30 D and 31 E, p. 651. It is interesting to note the occasional occurrence of outgrowths, W′ and X′, which appear to be rudimentary needles in the axils of the leaves marked $l'_{\cdot 1}$ (Figs. 31 C and D).

This brief account of the leaves associated with needles leads, I think, to the conclusion that the needles W and X are axes, on which one, two, or three vestigial leaves are developed, whereas the remaining needles of the group, probably because they suffer more from crowding than W and X, fail, as a rule, to develop leaves. Of the leaves borne by W and X, the leaf *pr.* in

Figs. 30 E, F, G, and 31 F, p. 651, is the one which is most liable not to develop; it is absent, for instance, in the needle-group shown in Fig. 32, in which $l'_{.1}$ and $l'_{.2}$ were present, and also in Fig. 33 B, in which $l'_{.2}$ was found alone. Again, though *pr.* is well developed in connexion with W in Fig. 30 G, it is present only in a rudimentary form in connexion with X. In those cases in which it is present, it is the last of the three leaves to become detached. I think it is possible that *pr.* may, in reality, represent the prophyll of the shoot, whose axis is the needle W or X; its delay in freeing itself I suppose to be due to its compressed position between the needle to which it belongs and the other members of the group. The reasons which lead me to this conclusion are, in the first place, that *pr.* occupies the characteristic position for a prophyll, and, in the second place, that, in its relation to the rest of the shoot and in its delayed detachment, it corresponds to the phylloclade of *Myrsiphyllum asparagoides*, which I have interpreted as a prophyll (pp. 637-40). In this connexion, Fig. 7 B, p. 636, may be compared with Fig. 31 F; in both it will be seen that the prophyll, *pr.*, remains attached to the main axis, after the leaves $l'_{.1}$ and $l'_{.2}$ have become free.

The discovery of the fact that leaves may be borne by the needles of *Asparagus* seems slightly to incline the balance to the 'vegetative shoot' theory of the needles, rather than the 'pedicel' theory; for Goebel has treated the alleged leaflessness of the needles as favouring the pedicel theory (16).

(v) *The Comparison of Myrsiphyllum and Asparagus.*

If the homologies outlined in the preceding section of this paper be admitted, the comparison with *Myrsiphyllum asparagoides* helps, I think, towards the understanding of the needle-fascicle of *Asparagus*. In *M. asparagoides*, the axillant leaf, *l.*, subtends one lateral branch, which may bear a single overgrown prophyll (phylloclade) and may then grow on into a terminal bud producing a series of leaves ($l'_{.1}$, $l'_{.2}$, $l'_{.3}$), which stand at right angles to the prophyll and the axillant leaf. *Asparagus Sprengeri* differs from *Myrsiphyllum asparagoides* in the fact that the axillant leaf subtends a number of shoots (needles) instead of one, and these—in lieu of bearing hypertrophied prophylls (phylloclades), followed by a succession of leaves—bear either no leaves at all, or else one or two reduced leaves with, sometimes, a vestigial prophyll in addition. It seems as if the energy, which in *Myrsiphyllum* goes into the development of an exaggerated prophyll, in *Asparagus* finds its outlet in the multiplication of lateral axes.

I am inclined to think that the arrangement of the leaves in *Asparagus* indicates that the needle-fascicle is not a reduced and telescoped branch system, but consists of a multiple set of buds, which may possibly, however, produce further needles of higher orders from the axils of their basal leaves.

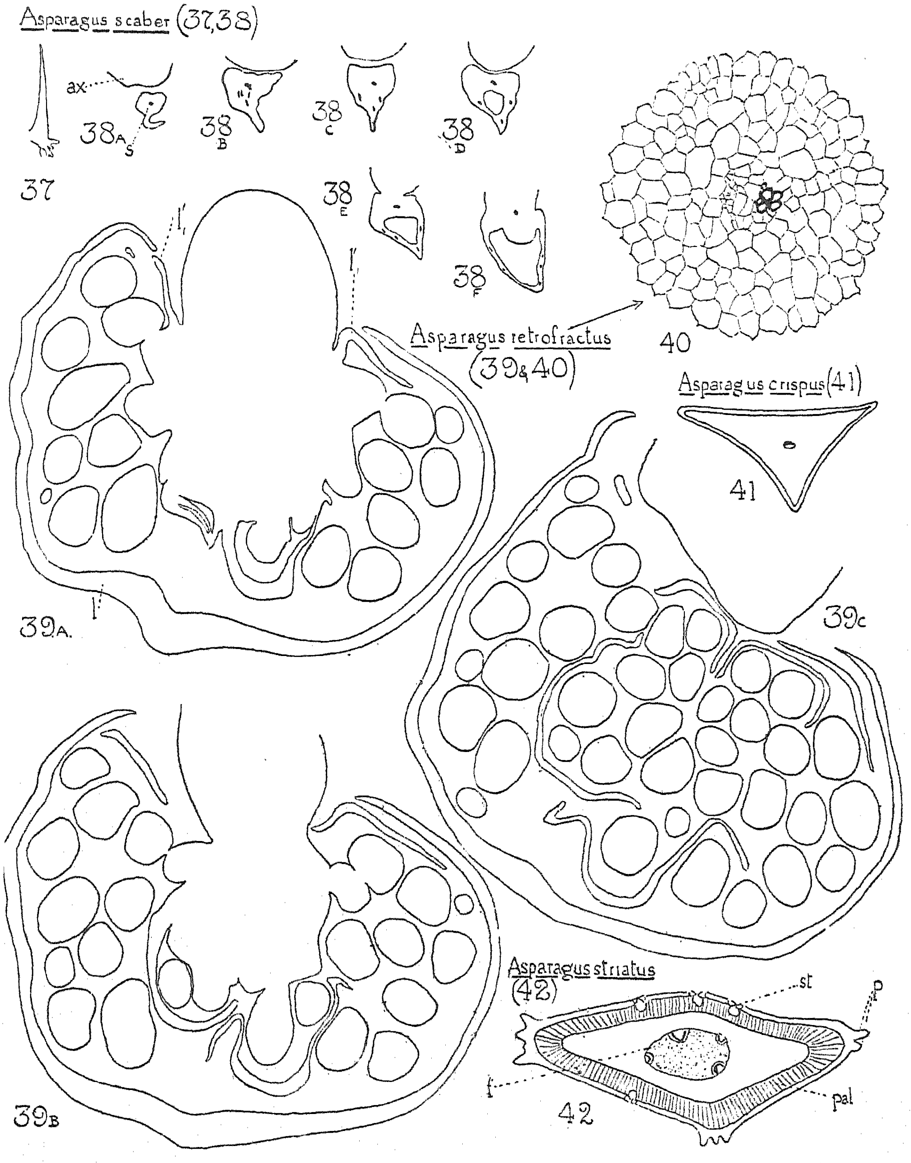
No exact analogy for this arrangement can be found elsewhere, but the series of buds which arise within a single scale-leaf of certain bulbs suggests a parallel condition.

(vi) *The Anatomical Structure of the 'Needles'.*

From a study of the relations of the needles as members of the shoot, we have been led to regard them as axial structures; it now remains to be seen whether the probability of this view is increased or diminished, when we take their internal structure also into consideration.

The vascular system of *Asparagus* needles consists of a single central cylinder, which usually includes a number of strands arranged in an irregularly radial fashion and often embedded in fibres. Figs. 43 A and D, p. 657, show the section of a needle of *A. falcatus*, L., which is traversed by seven bundles; *A. trichophyllus*, Bunge, has a similar cylinder (Fig. 13, p. 644), while the structure of *A. striatus*, Thunb., is also of the same type. I wish to draw attention to the latter species, because Reinke (20) has described the cladode as having two series of vascular bundles, lying just within the upper and lower palisade parenchyma. If this description were correct, the structure of *A. striatus* would be entirely unlike that of any other *Asparagus*. I have found, however, on examining a few needles from Boschberg (British Museum Herbarium), that the anatomy is, in reality, of the type familiar in the genus; Fig. 42, p. 655, shows the structure of one of these needles, while two others, of which I cut serial sections, had also precisely the same anatomy, with four bundles in the cylinder. It thus seems probable that the material which Reinke examined was misnamed. Velenovský (24) takes the view that the foliaceous needles of *Asparagus Sprengeri*, Reg., and *A. falcatus*, L., form a transition to the phylloclades of *Myrsiphyllum*. It is hardly necessary to discuss this suggestion, since it is at once put out of court by a comparison of the structure of these organs, as shown in Figs. 34 and 35, p. 651, Figs. 43 A and B, p. 657, and Figs. 4 A and B, p. 636. The needles of *Asparagus*—even when their form is somewhat leaf-like—are built on a fundamentally different plan from the phylloclades of *Myrsiphyllum*.

Mature needles of *Asparagus officinalis* have, at the base, three or four bundles in the cylinder (Figs. 23 A–C, p. 649); the tissue between these bundles is sclerized. Higher up, the number of bundles may be reduced from four to two (Figs. 24 A and B). The full equipment of bundles does not appear simultaneously in the very young needles—at an early stage one lignified bundle can sometimes be recognized, with one non-lignified procambial strand opposite to it (Fig. 21, p. 648), the other bundles being of later development. The additional bundles are not, however, branches arising from the first-formed bundles. The middle needle of Fig. 23 A, p. 649, for instance, can be traced without obliquity to its extreme base, and,



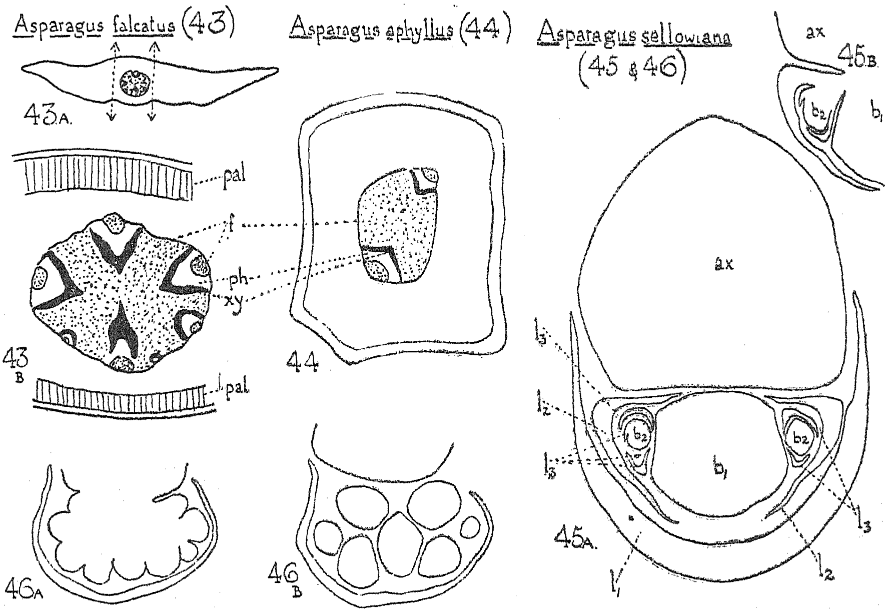
FIGS. 37–42. Figs. 37, 38, *Asparagus scaber*, Brign. Fig. 37, side view of scale-leaf with basal spine, which, in this species, bears emergences (nat. size). Figs. 38 A–F, transverse sections from series from below upwards through base of a scale-leaf; *s.*, spine (× 14). Figs. 39, 40, *A. retrofractus*. Figs. 39 A–C, transverse sections from series through the structures axillant to a single leaf, *l.* (× 47). Fig. 40, transverse section of needle (× 318) to show single bundle. Fig. 41, *A. crispus*, Lam., transverse section of needle (× 23). Fig. 42, *Asparagus striatus*, Thunb., transverse section of needle from Boschberg (Brit. Mus. Herbarium) (× 47); *f.*, central fibrous mass in which four bundles are embedded; *st.*, stomates; *pal.*, palisade parenchyma; *p.*, papillose epidermis.

from the first, all its four vascular strands are found to be independent. In *Asparagus Sprengeri*, the bundles have a similar history. In one group of four mature needles of this species, of which I cut serial sections, three of the needles had four vascular strands, while the fourth had three; in each of these needles, all the bundles remained free from one another to the extreme base. But in this species, as in *A. officinalis*, the vascular system of the young needles is less complex; they are characterized by a pair of bundles facing one another (Figs. 32 and 33, p. 651). In the case of the needles W and X, these strands lie in the plane of the leaves $l'._1$ and $l'._2$ (Fig. 32). In *A. trichophyllus*, again, the development seems to follow a parallel course, for here the young needles (Fig. 12 C, p. 644) have fewer bundles than the mature needles (Fig. 13), and one of the bundles generally seems to take the lead in lignification.

The needle anatomy of many members of the genus is reduced in comparison with the species which we have described. *A. aphyllus*, L. (Fig. 44, p. 657), has two bundles, while the delicate needles of *A. retrofractus* (Fig. 40, p. 655) and *A. crispus*, Lam. (Fig. 41), have a single strand; in the latter species, the small bundle is embedded in trabecular mesophyll.

The features in the anatomy of the needles to which attention has been briefly drawn are, I think, on the whole favourable to the axial interpretation. But it may, on the other hand, be suggested that a cylinder with a slightly irregular radial anatomy, such as that found in *Asparagus* needles, is not necessarily axial, since it may be paralleled, even within the Liliaceae, among organs that are undoubtedly foliar, e. g. the leaf of *Laxmannia* (2, pp. 454–5, Figs. 19 C and 21). The fact that, in the early stages, the needles may have only one or two bundles may also be cited in this connexion. But I think these arguments are outweighed by the fact that the mature needle of *Asparagus* shows no definite dorsiventral symmetry about any plane which can be related to an axis—existent or hypothetical; the vascular skeleton of a leaf, on the other hand, invariably has a definite orientation in relation to the parent axis.

If it be agreed that the anatomy of the needles supports the axial theory, the next step is to compare them with the pedicels, in order to see whether the anatomy lends support to the view that the needles can be assigned to one particular class of axis—the flower-stalk. If Fig. 36 D, p. 651 (the upper part of the pedicel of *A. Sprengeri*), and Fig. 34 (the basal region of a mature needle of the same species) be compared, it may be admitted that there is a similarity of structure which suggests that these organs are equivalent; but it should be noted that sometimes the pedicels are less sclerized than in the cases figured, so that the resemblance between the two organs becomes less striking. It is possible, indeed, from this one example to get an exaggerated idea of the general degree of resemblance between pedicels and needles. In *A. officinalis*, for instance, the pedicel, even when quite young, has a ring of procambial strands (*p.*, Fig. 25, p. 649), while the young needle has, as we have seen, only one or two bundles (Fig. 21, p. 648), the radial scheme being completed later. The mature pedicel (Fig. 22, p. 648) is also by no means the exact counterpart of the mature needle (Fig. 24 A, p. 649). It thus seems to me that the vascular scheme of the needles of *Asparagus* presents no such precise similarity to that of the pedicels, as to make it necessary to assume that the needles are sterilized flower stalks.



FIGS. 43–6. Fig. 43, *Asparagus falcatus*, L. Fig. 43 A, transverse section of a needle (×14). Fig. 43 B, the part of Fig. 43 A, between the arrows (×77); *pal.*, palisade parenchyma; *xy.*, xylem; *ph.*, phloem; *f.*, fibres. Fig. 44, *A. aphyllus*, L., transverse section of needle (×77). Figs. 45 and 46, *A. sellowiana.* Fig. 45 A, transverse section of turion (×47) to show, in axil of leaf $l._1$, lateral bud $b._1$, itself bearing leaves $l._2$ and $l._2$, with buds $b._2$ and $b._2$ in their axils, bearing leaves of the third order, $l._3$. Higher up, it is found that the bud in the axil of the third leaf borne by $b._1$ has needles in the axil of leaves of the order of $l._3$. Fig. 45 B, section through $b._2$ at a level a little below that of Fig. 45 A, to show attachment to axis (×47). Figs. 46 A and B, two transverse sections from a series through a leaf (probably corresponding to $l._3$ in Fig. 45) and its axillary needle-group (×77).

The sectional outline of the needles is curiously variable. They may be cylindrical (e.g. *A. retrofractus*, Fig. 40, p. 655), triangular (e.g. *A. crispus*, Fig. 41, p. 655), or of an irregular, slightly angled form (e.g. *A. officinalis*, Figs. 24 A and B, p. 649, and *A. aphyllus*, Fig. 44, above); or they may be winged and foliaceous (e.g. *A. falcatus*, Fig. 43 A, above, and *A. Sprengeri*, Figs. 29 and 35, p. 651). In the case of *A. Sprengeri*, I have observed the curious fact that these wings originate in the plane of the two first bundles, which is also that of the leaves $l'._1$ and $l'._2$ (Figs. 32 and 33 C, p. 651). Now the winging of stem structures is not a casual phenomenon, taking place fortuitously in any plane, but is a development of decurrent leaf-bases, and hence is related to the orthostichies (3, pp. 308–10). I should like to suggest that the wings of the needle of *Asparagus Sprengeri* (and possibly of other species with leaf-like needles) may be the last indication of the decurrent bases of leaves, whose free part has ceased to exist, but which once formed a distichous series, of which only the two basal members, $l'._1$ and $l'._2$, have survived as non-vascular scales. This conception would be in harmony with the leaf-skin theory of E. R. Saunders (22). The two opposite bundles shown in each needle in Fig. 33 C, p. 651, &c., may also owe their precocious appearance, and their situation in this definite plane, to the fact that they represent the strands which originally supplied two ranks of leaves.

5. SUMMARY.

In the present paper, which forms a sequel to a recent discussion of the Rusceae (5), an attempt is made to interpret the morphology of the 'phylloclades' of *Myrsiphyllum* (sometimes treated as a sub-genus of *Asparagus*); the spinous scale-leaves of *Asparagus*; and the 'needles' of *Asparagus*.

It is concluded that the 'phylloclades' of *Myrsiphyllum asparagoides*, Willd. (*Asparagus medeoloides*, Thunb.), are the prophylls of lateral shoots. In the majority of cases, the axes of these lateral shoots are abortive, and produce no other organ than the prophyll, which is precisely comparable with the phylloclade of *Danae* among the Rusceae (5). But the reduction of the prophyll-bearing shoot has not reached so extreme a point as in *Danae*, and it sometimes continues to grow, again producing a series of scale-leaves with axillary phylloclades. The prophyll interpretation of the phylloclade is extended to the three other species of *Myrsiphyllum*, and attention is called to an anatomical resemblance between the phylloclades of *M. undulatum*, Schlecht., and those of certain Rusceae.

The spinous scale-leaves of *Asparagus* are regarded, in accordance with Buscalioni's view (9), as ligular sheaths, the downwardly directed spine or tail being taken to represent the petiolar limb.

The 'needles' of *Asparagus* are held to be axial. The chief piece of new evidence brought forward in support of this contention is the fact that the needles sometimes bear as many as three small scale-like leaves in their basal region—a fact which seems to have been hitherto overlooked. But though the cladode theory is accepted, it is held that there is no conclusive evidence for the view that the needles represent sterilized pedicels, rather than vegetative shoots.

BALFOUR LABORATORY,
CAMBRIDGE.

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The Root-tubercles in Arbutus Unedo.

BY

M. F. RIVETT.

With fourteen Figures in the Text.

IN 1917 a paper appeared entitled 'The Endotrophic Mycorrhiza of the Ericaceae', by Jean Dufrénoy (2). It deals mainly with *Arbutus Unedo* and its endophyte, the material being obtained from plants growing wild in certain districts of the south-western region of France. The general relationship between host and endophyte is briefly indicated; the existence of root tubercles is noted and some description of their growth put forward, but the chief points of interest in regard to these organs are omitted. I have therefore endeavoured, at the suggestion of Dr. Rayner, to add to those of Dufrénoy further observations on this plant and its endophyte, using as material plants growing in pots in a compost of loam, sand, and peat. These were raised as seedlings by nurserymen, some in England and some in Ireland, and varied in age from one to five or six years.

The Main Roots of the Plant and their Relation to the Endophyte.

Dufrénoy states that the roots of *Arbutus Unedo* are clothed with a dense mantle of hyphae. This is not always to be observed in my material. The older roots shed their epidermal covering, and develop a corky bark which is not conspicuously invaded by hyphae. Also in many of the young roots, white in colour and without a corky covering, the surface may be comparatively clean. Frequently, however, there is a limited growth of hyphae on the younger long roots, both of the endophyte and of numerous epiphytic soil fungi, such as *Alternaria*. The latter may be found fruiting abundantly on the surface of young roots. In either case (whether an outer investment or apparent ectotroph is present or not) all the roots examined in detail show the existence of a mycorrhizal fungus, varying in its extent and development. This can easily be observed a month or so after fresh root-growth begins. The newly-formed roots of plants potted in February begin to break through

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the soil as they reach a length of four or five cm. during May and June. If examined in lactic phenol after staining with cotton-blue in lactic acid, both these roots and their small secondary branches show a limited growth of the endophyte. In the majority of such roots the hyphae are closely applied to the outer surface, growing as long strands and loose irregular coils, very rarely forming close knots or densely interwoven branches. Many of the long strands run in the longitudinal walls of the epidermal tissue, others straggle around the root in loose uneven spirals. They are sparingly branched, and what branches they have are long and spreading. The diameter of the hyphae is 4–8 μ; the outer walls are thick and stout and stain with Sudan III; there are numerous cross-walls, also fairly thick; the segments are multinucleate and show a granular, protoplasmic content.

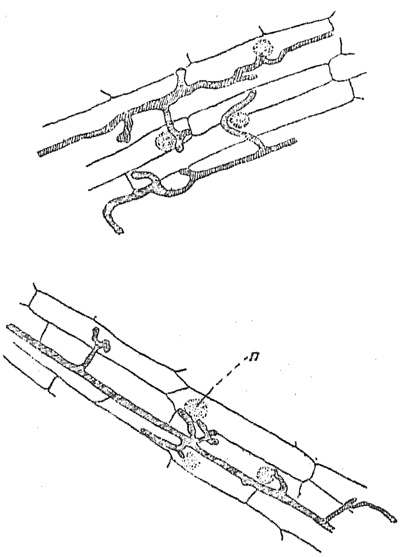

FIG. 1. Young mycelium on surface of main root: branches just entering the cells. *n.*, nucleus.

In a small minority of roots the fungus is more profusely developed. Beyond the closely applied hyphae there are many more which grow away from the surface and branch more freely. They are not sufficiently dense to form a covering visible to the naked eye, but, when stained with cotton blue, show up as a loose collection of entangling threads. These hyphae are narrower, and have thinner walls than those which are closely applied to the surface. Moreover, the roots with which they are associated are rather attenuated and mucilaginous, contrasting in appearance with the thicker, more vigorous roots on which the growth of the fungus is limited to closely applied hyphae. The obvious conclusion is that these roots are abnormal and are parasitized by the fungus, instead of maintaining a resistance against its growth.

Returning to the normal, resistant roots we find that branches from the exterior may pass through the outer wall into the cavity of the epidermal cells, though not in every case. This wall may be either of cellulose or suberized; it is difficult to determine whether the fungus obtains an entrance in the young state of the epidermis before suberization occurs, or enters afterwards in spite of the suberization; probably the former is more common. In the cavities of the outermost cells there are short hyphal branches, resembling haustoria; they may give off two or three further branches with blunt tips, or a more abundant series of finer threads, spreading throughout the cavity. In either case, branches are frequently to be found in intimate association with the nucleus of the cell (Fig. 1). A general inspection of the superficial layer as a whole does not give the impression that it is abundantly invaded by the endophyte, though probably the majority of the cells show traces of hyphae. Within the epidermis is the suberized layer with comparatively thick walls; hyphal branches run in between the cells of this layer, but only here and there, traces being difficult to find; they rarely enter the cavities of the cells. In the deeper-seated layers of the cortex, the hyphae are considerably attenuated and still more difficult to find, though they can occasionally be seen running in between the longitudinal walls and still more rarely entering the cells. Up to the present I have not been able to detect any traces within the endodermis, though hyphae may be present in a still more attenuated form.

From this short description it is apparent that the intracellular development of the endophyte in the main roots is slight, and this will become more obvious when it is compared with the intracellular development in the more specialized root-tubercles. It is here, in the normal main roots, a weakly growing parasite, easily kept in check by the host without evident disturbance in its metabolism or reaction in its tissues. In its superficial appearance, i.e. in its method of growth in strands and coils, it bears some resemblance to the typical mycorrhiza of other members of the Ericaceae.

The Morphology and Growth of the young Tubercles.

Five or six months after the earliest renewed root-growth, in late February or March, the first young laterals begin to break through the parent cortex, frequently encased in a sheath of transparent mucilage. M. Dufrénoy states that the mucilage is brought about adventitiously by external bacteria and algae, but its presence on comparatively clean roots is inconsistent with this view, and there is some evidence that it is a natural feature of the root system, and particularly associated with lateral root-development both in *Arbutus* and in other members of the Ericaceae. The extent to which the mucilage is produced varies from time to time. It seems always to be present as a very thin film over the surface of the main roots, and this is pushed out and distended as the incipient rootlets emerge. In some laterals it becomes conspicuous as a sheath, in others it can hardly be detected, but throughout the growth of many roots it is to be found around the growing-point. It has been suggested that when the mucilage becomes conspicuous it is rather in the nature of a pathological reaction, due to lack of moisture in the soil. Mucilage is known to act as an agent retentive of water, and it may be that the young roots are protected in this way against drought. The suggestion receives some confirmation from the state of the roots in potted plants kept fairly dry. The mucilage blackens slowly with osmic acid, but does not stain with Scharlach red or Sudan III; it stains with eosin, methylene blue, and Bismarck brown; its character is probably mainly pectic and partly oily.

Normal growth is usual in the first-formed laterals, but many of those arising successively may have their growth partially checked or arrested by a more intimate and profuse development of the endophyte. On a great number of the emerging rootlets the fungal hyphae begin to grow with exceeding vigour, forming a dense outer covering of hyphae six or seven layers thick, and soon penetrate into the interior, where they react with the host tissues, with the result that very shortly after its emergence from the parent cortex a rootlet so infected is converted into a rounded or pear-shaped tubercle, swollen at its distal end. Such young tubercles are shown in Figs. 2 and 3. They can be recognized by their white colour and peculiar shape when only 0·2 mm. in length, but they may grow without branching up to 4 mm. or more. On the other hand, lateral roots may grow normally up to 2 cm. or more in length, when their growth may be arrested and their tips develop into tubercles.

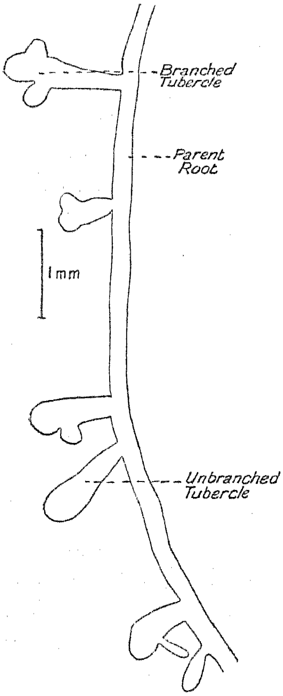

FIG. 2. Branched and unbranched tubercles.

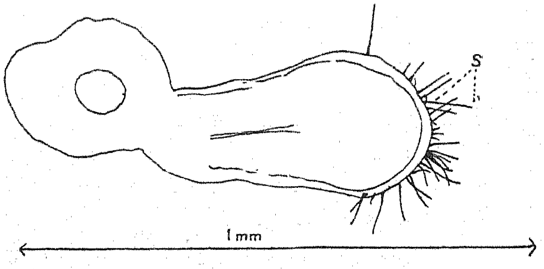

FIG. 3. Very young tubercle with setae, S.

The Endophyte in the Young Tubercles.

The abundant hyphae forming the outer investment of the tubercles are very characteristic; they are large in diameter ($5-10\mu$) and are made up of short segments with thick transverse walls; the outer walls stain with Sudan III; they have abundant protoplasmic contents and more than one nucleus to each segment. These hyphae do not grow in straight threads or in loose spirals as do those on the long roots, but form a very great number of short branches and curved, looped segments, which interweave with one another to form a close investment in which no continuous hypha is visible, and which has the appearance of a pseudo-tissue of irregular cells with clear or granular cavities and rather thick walls. The method of growth of the hyphae somewhat resembles the process of budding. Short, almost spherical branches appear at first, and just after these have begun to elongate, they branch again and so on. The investment formed in this way may have a thickness of six or seven layers of hyphae. Between them is the mucilage derived from the surface of the root. To this gritty soil particles adhere, and the whole forms a coating difficult of penetration by xylene and paraffin, and extremely difficult to section by means of a microtome.

The formation of this outer investment and the establishment of an ectotrophic condition is interesting in the light of statements made by previous writers in regard to the mycorrhiza of the Ericaceae. Gallaud remarked that he did not study the mycorrhiza in this family because a first inspection led him to think that the form was ectotrophic—a rather surprising view, in the light of what we have more recently learnt about *Calluna*. However, it may be that he did not examine *Calluna*, but only arboreal forms such as *Arbutus*, in which the mistake would be pardonable. At the same time, it is to be noted that the distinction between ectotrophic and endotrophic forms can now only be regarded as rough and arbitrary, and *Arbutus*, as will be seen, affords a good example of the transition between the two.

It is undoubtedly the successful early establishment of the ectotrophic condition that determines the future tuberization of the emerging rootlet and prevents the continuance of normal root-growth. In examining young laterals as they emerge from the cortex, we can see that some have only a few hyphae applied to their surface, and that these are of the straight, long-growing variety found on the parent roots; such laterals will continue their growth. In others the hyphae are abundant, and the investment can be seen beginning to develop owing to the continuous formation of short branches which interweave; such laterals will develop into tubercles. It was suggested by M. Dufrénoy that infection of the tubercles took place from the soil, but after examining a great number of roots with incipient laterals, I have concluded that many of the tubercles are infected from the main root. Even the pushing out of the cortical cells and the extra formation of mucilage seem to stimulate the hyphae in the vicinity, and if they are present in quantity they surround the point of emergence of the rootlet and begin to weave their mat-like growth.

Another curious feature of the external investment in a number of the young tubercles is the development of some of the hyphae into prolonged setae, reaching 0·1 mm. in length (see Fig. 3). They arise as outgrowths from the outermost hyphae of the superficial layer and grow at right angles to the surface of the tubercle. They have thick external walls at the base, and well-marked transverse walls, and are comparatively rigid. They taper towards the apex, and thus have the appearance of radiating spines. They are similar to the setae in figures illustrating the parasitic fungus *Colletotrichum*. It is comparatively difficult to trace the junction of the setae with the closely applied hyphae of the investment, and I was only successful on two or three occasions. I have shown this junction in Fig. 4, and regard the fact as established. For some time I had regarded these setae as root-hairs, emerging from the host tissue of the tubercle, having been influenced by M. Dufrénoy's statement that 'nearly all the epidermal cells of the tubercle develop into root-hairs'. As a matter of fact, nearly the opposite is true, and none of the cells develop into root-hairs, these organs being absent in *Arbutus* and in other genera of the Ericaceae. It may be that the setae function as absorbing organs for the fungus, and provide the superficial investment with soil moisture, but it is not improbable

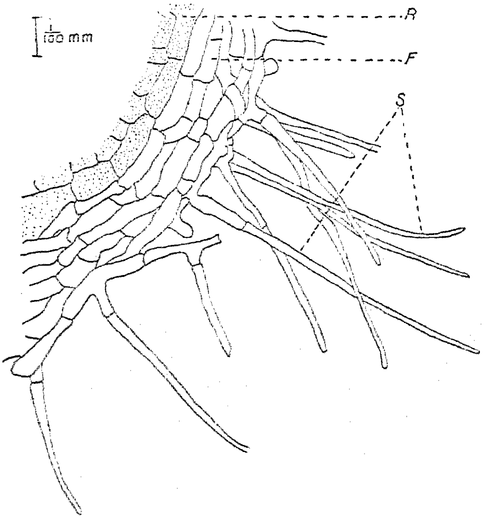

FIG. 4. S., setae; F., exterior fungal hyphae; R., root-cap cells of host.

that they are characteristic morphological features, which could be recognized as such in pure culture. The setae are only found on very young tubercles, and not always on those, but when found, they are striking in appearance. Their resemblance to root-hairs is superficial, the pointed tip and thick basal wall being the distinguishing characteristic.

It is possible that the mechanical pressure of the close investment of hyphae may have some preliminary influence on the growth of the tubercle, but its formation is very rapidly followed by the penetration of hyphae into the epidermal cells, which at once produces change of habit. The early stages of infection have been gathered from various separate observations, which may be pieced together as follows: in many cases numerous hyphae penetrate the thin walls of the epidermis immediately behind the growing-point, before the cells have reached maturity. Each infecting hypha grows into the cavity of the cell and begins to coil and branch, but it cannot be observed as a distinct thread, because the host cells immediately react and cause the threads to 'clump' and coalesce, to lose their distinct outline, and to become very quickly converted into a darkly staining body which occupies the middle of the cavity of the cell and adheres to the nucleus. This is undoubtedly the almost completely digested mass of the contents of the hyphae. A similar reaction is described by Noël Bernard in his work on the orchids in the words: 'Le peloton entier est digéré par la cellule hôte et se réduit à une masse de dégénérescence amorphe.' The rapidity

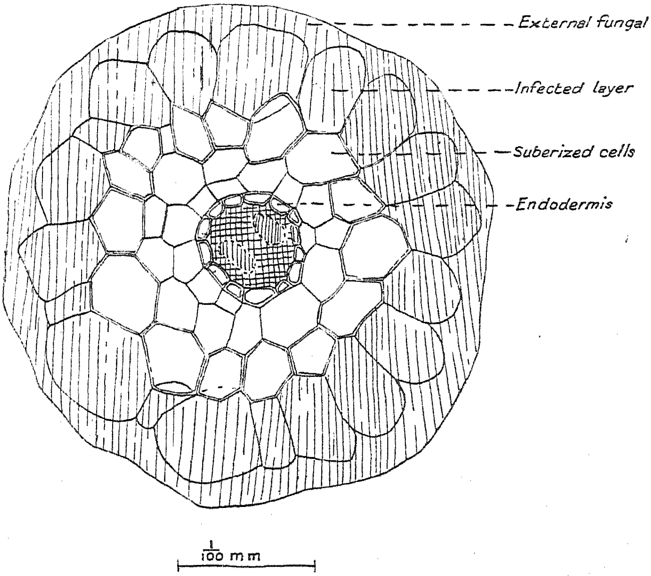

FIG. 5. Transverse section of a young tubercle.

and completeness of the reaction in these cells of *Arbutus* may be ascribed to their youthful condition. At the same time the disturbance to metabolism involved in the process of digestion inhibits the normal activity of the growing-point and causes an enlargement in the infected cells. In other words, it is the physiological phenomenon of intracellular digestion which produces the morphological effect of tuberization.

It is not in every tubercle that infection takes place so close behind the growing-point, though such is frequent enough. Young tubercles can be observed in which there is no digested mass in these cells, because the first infection has taken place a little lower down. There is a difference in the form of the tubercle corresponding with this difference in infection; those more apically infected have a thicker head than the others.

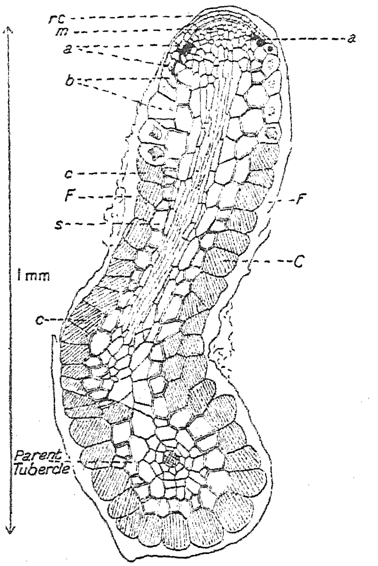

FIG. 6. Longitudinal section of a young tubercle: *r.c.*, root-cap; *m.*, meristem; *a.*, first infected cells (enlarged in Fig. 7, *a*); *b.*, cell with contents completely digested and large granular nucleus; *c.*, reinfected cells; F., external hyphae; *s.*, suberized layer.

Cell-division continues to take place slowly in the growing-point, accompanied by infection of the young cells; at the same time the first infected cells increase abnormally in size as they pass over into permanent tissue. Here intracellular digestion and absorption may proceed so rapidly that they are entirely emptied of fungal contents, and the cells appear normal except for their large size and the condition of the nucleus. This is of great dimensions, very granular, frequently distorted in shape. The membrane is inconspicuous or invisible owing to a closely applied coating of cytoplasm, and adhering to this are wisps and fragments of digested material, which can, in a few rare cases, be connected with unaltered or partially altered pieces of hyphae situated in the peripheral part of the cell. No immunity is conferred on the cell by the process of digestion, and reinfection may take place either before it is complete or very soon afterwards. Reinfection takes place by the inward growth of one or more of the thick-walled hyphae forming the superficial investment. These often penetrate along the radial walls of the epidermal-like layer, apparently dissolving the middle lamella and pressing the cellulose layers apart. From this position the tip of the hypha passes through the cellulose layer into the cavity, where it coils and branches irregularly. It remains unattacked long enough for its form of intracellular growth to be studied.

For the purposes of comparison it may be worth while briefly to recall the intracellular mycelium described by previous writers. Bernard, in his work on the orchids, describes coils or balls formed by an apparently continous hypha; this is not much tangled or knotted, but wound round and round in a loose ball; there is little or no branching, but a continuous coiling growth. Such formations are described as 'pelotons', and occur both in pure culture and in association with the host plant. Gallaud describes a rather different formation occurring in *Arum maculatum* and a number of other species. Here a stout trunk hypha enters the cell and branches considerably, forming series of twig-like growths of continually decreasing diameter: the whole may be complex and extensive, filling the cell with these twig-like branches, which more evidently resemble haustoria than does the 'peloton' of Bernard. Gallaud uses the word 'arbuscule' to signify this type of intracellular mycelium.

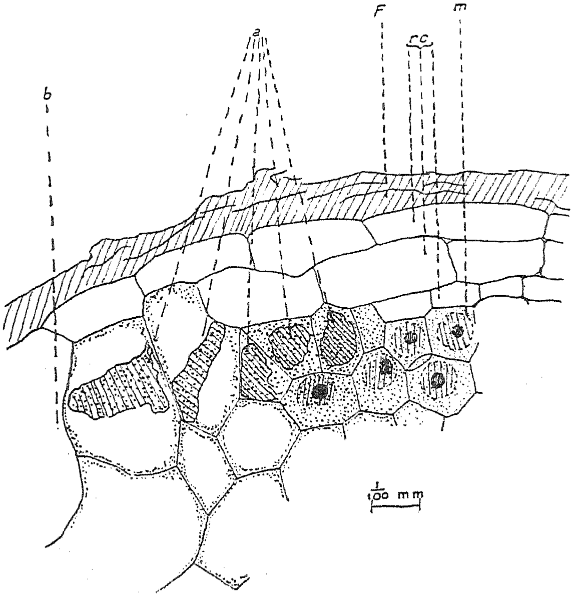

FIG. 7. First stages of infection: *m.*, meristem; *r.c.*, root-cap cells; F., external fungal hyphae; *a.*, first infected cells with 'corps de dégénérescence' (cells marked *a* in Fig. 6 more highly magnified); *b.*, cell emptied of digested contents; *r.c.*, root-cap cells.

In *Arbutus* I have seen no such regular coils as the 'pelotons' of Bernard, nor anything approximating closely to the twiggy 'arbuscules' of Gallaud. Rather, the hypha forms a loose and irregular tangle, somewhat sparingly branched: owing to the large size of the host cell, it cannot be obtained complete in a single section suitable for study under a high power of the microscope. I would say that in so far as the loose growth of the tangled hypha does not suggest a haustorium, it resembles a peloton rather than an 'arbuscule', though sometimes a stout trunk-like hypha just within the cell-wall suggests the base of an 'arbuscule'. However, I think that the biological significance of these varied forms of intracellular mycelium is very much the same, the morphological differences being due to the specific nature of the fungus and the host, rather than to any physiological difference in their methods of reaction: and in this view the multiplication of names to express differences in form becomes unimportant.

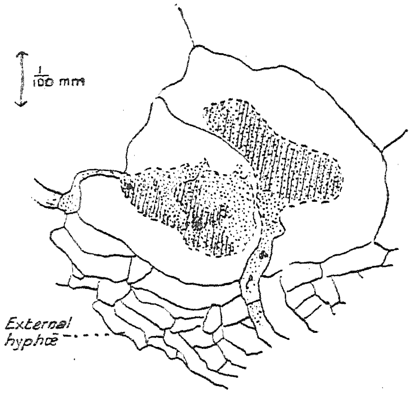

FIG. 8. Semi-digested mass of hyphae resembling 'sporangioles'.

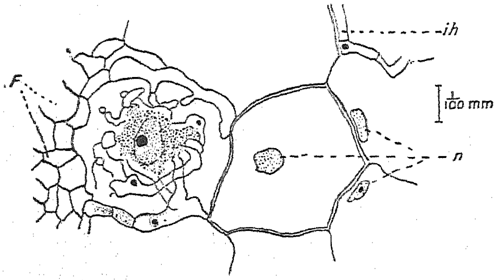

FIG. 9. Reinfected cell. F., external hyphae; *i.h.*, intercellular hypha penetrating into cortex; *n.*, nuclei of uninfected cells.

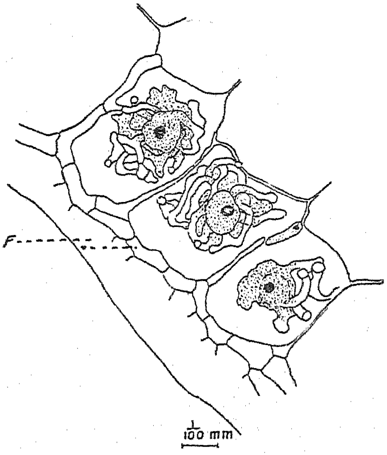

FIG. 10. Reinfected cells. F., external fungal hyphae.

Branches from the tangle may pass out on the inner side of the wall and grow between the walls of the cells of the underlying suberized layer, dissolving the middle lamella as they go. I have not observed hyphae in the cavities of these cells, but they may reappear very occasionally in the cavities of the more deeply situated cortical cells, though here they never develop in any profusion.

Digestion continues to take place after reinfection, though not following so quickly as in the more apical cells. The appearance of the partially digested hyphae varies from cell to cell. In some the infecting hypha from without the cell can be seen passing through the wall and into the cavity as a trunk or stalk, devoid of contents; it terminates in a tangle of coiled hyphal remains, rather coalesced, granular, embedded in a kind of mucilage which stains deeply and blurs the outlines. This rather resembles the 'sporangiole' of Gallaud, although he applies the term to the mass resulting from an 'arbuscule', but it is the same kind of semi-digested mass, formed from the membranous walls of the endophyte, not assimilated by the host cell, dead, inert, without a further rôle. (See Fig. 8.)

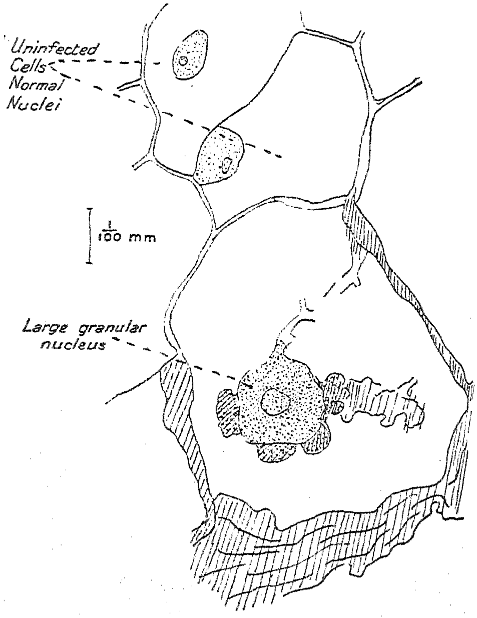

FIG. 11. Cell with hyphae nearly digested.

In other cells the coiled and branched hypha can be seen more clearly: towards the outer limit of the cell the walls of the hypha are well defined, but the cavity is clear, entirely depleted of protoplasmic contents; in the midst of the cell the branches are more 'clumped' and the walls are partly broken down, while abutting on the nucleus there are granular mucilaginous masses of completely disintegrated hyphae (Figs. 9, 10).

In all these cells the nucleus is of large dimensions and often of irregular form, pear-shaped or amoeboid in outline (Figs. 9, 10, 11). The nucleolus is large and conspicuous. Occasionally there are two nuclei in the same cell, closely apposed. Such digestive nuclei may be from 10 to 14 μ in diameter, while in the uninfected cells they are from 7 to 9 μ. Similar nuclear phenomena have been described by other workers. Gallaud reports the multiplication of nuclei in the host cells containing 'sporangioles', and Bernard describes and figures in *Phalaenopsis* and other orchids enlarged and deformed nuclei, which he regards as phagocytes, predestined for this function.

In some tubercles, where digestion is proceeding actively, the fungus is very abundant on the exterior, forming not only the closely applied, dense superficial investment, but producing also long branches of straggling, branched hyphae, of narrow cross-section and with a thinner external membrane; they have numerous cross-walls and may bear simple solitary conidia. It may be that in this condition the fungus is extracting material from the interior cells, and that it also makes use of the mucilage which is abundant on such heavily infected tubercles. On the other hand, it may be that on these tubercles there is present another fungus besides the endophyte.

It is noteworthy that the tissues of the young tubercles are very mucilaginous as compared with those of the main root; both the walls and the cell contents appear shrunken and distorted, even after careful treatment with dehydrating agents. It is thus impossible to prepare permanent sections in Canada balsam which look at all natural. This is particularly true of those mucilaginous and highly infected tubercles just referred to.

The young tubercle continues to some extent to develop as a root, in so far as it acquires a vascular system of prosenchyma, wood vessels, and sieve-tubes, which connect with the conducting tissue of the main root. An endodermal layer surrounds the system: it has suberized walls which do not turn blue with sulphuric acid and iodine and which stain with Sudan III.

The endophyte during this development is still mainly limited to the peripheral cells, but a few inward-growing hyphae pass between the cells of the suberized layer of the cortex and can be distinguished here and there in the intercellular spaces and occasionally in the cavities of the deeper seated cells with cellulose walls. Digestion here is not so active as in the peripheral cells. The hyphae grow as far as the sheath surrounding the vascular system and here produce more intracellular branches: they are not abundant or conspicuous, but become partially digested and form small, deeply staining masses within the cells.

The relation between host and endophyte is depicted in Figs. 5–11.

The Reaction of the Endophyte and the Host to Stains and Reagents.

The reaction of the endophyte and the host results in a deposition of materials which can be detected by various stains and reagents. If tubercles are fixed in weak Flemming's solution and sectioned longitudinally, there is seen considerable reduction of the osmic acid by the tissues on a very definite plan (see Fig. 12). The peripheral layer of cell containing the partially digested fungus is blackened, and the internal contents of entire hyphae are also stained. The cells of the root-cap, forming a layer three or four cells deep, are filled with a material which is yellowish and highly refractive in the natural state and stains intensely black with the osmic acid. The endodermal sheath enclosing the vascular cylinder and the junction with the parent root are also deeply stained.

This stainable material which reduces osmic acid stains only faintly with Scharlach red and does not show the characteristic bright globules which this stain usually forms in the presence of oil. Nor does it react with Sudan III, except after prolonged warming with the stain in glycerine and alcohol, and then it is tinged red without the formation of bright globules.

Materials fixed for half an hour in Carnoy's solution, sectioned longitudinally and osmicated, shows the same blackening as that fixed in Flemming. Thus the stainable material is not readily removed by chloroform. Material passed through xylol and embedded in wax cuts exceedingly badly and provides torn sections, which still, however, blacken with osmic, showing that the material is not removed by xylol. When treated with a concentrated solution of Nile blue boiled with sulphuric acid, sections take up the stain instantaneously and are coloured deep blue; if treated with a weak solution of the same reagent, the regions staining with osmic acid show a reddish-brown coloration, faint in the peripheral cells but deeper around the growing-point and around the vascular system. Treated with ferric chloride, the same regions turn blackish-brown.

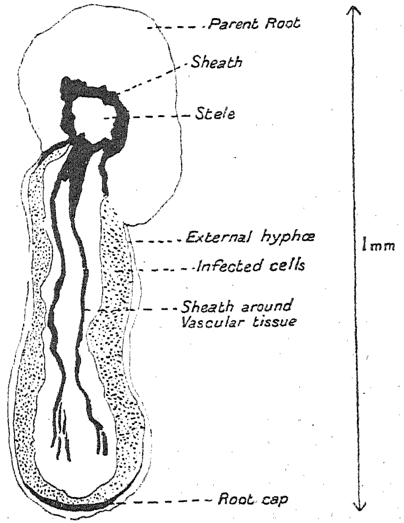

FIG. 12. To show distribution of stainable material. Longitudinal section of young tubercle.

These microchemical observations afford little clue as to the nature of the material which accumulates in the infected cells, the endodermis, and the root-cap. It therefore seems better, rather than to propose suggestions based only on staining reactions, to postpone the consideration of their chemical nature until more material is available for experimental work. In passing, it is of interest to note that stainable contents, probably of nutritive value, accumulate in the peripheral cells and in the endodermis of the tubercles, and that the supply of food to the growing-point may approach from a different angle from that in normal roots.

The further Growth of the Tubercles and their Morphology when Mature.

The young tubercles have at first one growing-point—the apical meristem of the lateral rootlet. It is only in rare cases that this ceases to function and becomes infected by the endophyte, but if it does occur, the tubercle remains as a simple club-shaped protuberance from the main root. More frequently the growing-point retains its activity and the tubercle increases in length, though the growth is very slow compared with that of a normal root. Sooner or later the growing-point divides and gives rise to two branches, exogenous and apparently dichotomous. The exogenous origin may be related to the peripheral food-supply consequent on the digestion of the fungus and may indicate a real absorption by the host from the endophyte. If so, it proves rather an interesting corollary to Professor Priestley's recent work on the function of the endodermis. He points out that the normal endogenous origin of roots is due to the early formation of an endodermis which prevents any passage of material from the stele to the cortex. In *Arbutus* we have the possibility of exogenous branching owing to the presence of a peripheral fungus.

The branches of the tubercle may grow to 2 or 3 mm. in length, or may themselves branch in the same way, and so on till the end of the growing season early in November. Thus by the end of the year the long roots bear numerous groups of extensively branched tubercles, most of the branches lying in the same plane and forming a characteristic, small, flat mass (see Fig. 13). Nearly all the branches develop conducting systems which connect together and with that of the parent root. The whole becomes dark brown in colour and firmer in texture. The outer hyphae shrink, the mucilaginous envelope dries, and the tissues have less tendency to contract during dehydration.

The Endophyte in the Old Tubercles.

Infection by the fungus keeps pace with the production of new cells by the growing-point, and digestion and reinfection proceed successively, Thus the peripheral cells, except at the growing-points, are to be found filled with partially digested hyphae. In many of the branches formed late in the season the hyphae penetrate more deeply into the cortical cells, which take on the same appearance as those at the periphery. More rarely the growing-point ceases to function and the whole tissue of a branch becomes infected, without any formation of vascular tissue. Digestion proceeds all the time that the tubercles are growing, and even in the winter, when the vitality of the host is at its lowest, it is difficult to find clearly defined hyphae, though here and there a cell may be picked out in which the hyphal walls are clearly defined (see Fig. 14). In the great majority of cells the cavities are filled with a granular mass of deeply staining material, in the midst of which persists a large nucleus. The endodermal sheath becomes densely filled with a mixture of fats and fatty acids, and also with crystals and grains of more solid matter: the conducting tissue itself also becomes blocked with materials which stain deeply with eosin, safranin, gentian violet, haematoxylon, and other reagents. The tubercles persist in this condition throughout the winter and the early spring. During the next growing season many of them are cast off, when the periderm is formed and cork cells are exfoliated.

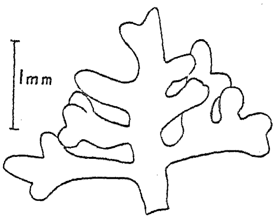

FIG. 13. Old branched tubercle.

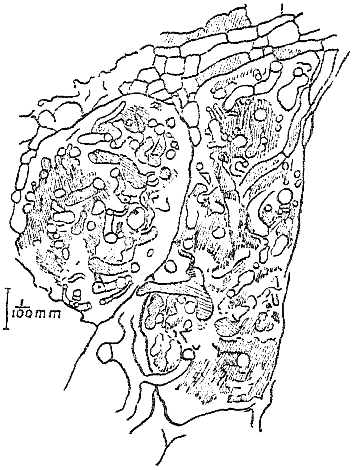

FIG. 14. Infected cells from old tubercle fixed in late winter.

DISCUSSION.

There are several points for discussion arising out of this consideration of the root-tubercles of *Arbutus Unedo.*

Following the order taken in the main body of the paper, the first point which arises is in connexion with the different form of growth in those hyphae on the roots and in those on the young laterals about to be converted into tubercles. What causes the long straggling growth in the one, and the short interweaving branches in the other? The former is the more natural method of growth for fungi on a homogeneous, dilute, nutritive medium, the latter more associated with symbiosis and parasitism. But though this is true about fungi in general, we do not yet know the specific fungus which invades *Arbutus,* and it will be necessary to see if this general statement holds good when it is grown, if that is possible, in pure culture.

At this stage I am inclined to say that the growth of the fungus on certain emerging laterals is influenced by the greater production of mucilage and by the excretions from the ruptured cells of the parent root, but this influence only takes effect if there are vigorously growing hyphae in the immediate neighbourhood. The characteristics of the fungus in this medium (the inability of the branches to lengthen, their tendency to broaden and branch, and their close apposition to the host surface) all tend to localize the fungus with regard to the host, and this localization seems to be the first factor in a series of reactions which has led to the regularization of the growth of the invader and to the conferring of some degree of immunity (as compared with the tubercle) on the main roots. It seems to me that this interweaving form of growth in the *Arbutus* fungus is directly comparable to the 'pelotonnement' of the Rhizoctonias, of which Bernard says, 'La clef du problème de l'immunité dans la symbiose doit être dans la découverte des conditions qui déterminent la formation des pelotons myçéliens'. It will be remembered that Bernard suggests a comparison of this phenomenon with that of agglutination in the bacteria.

The problem of the mechanism of the entrance of the endophyte into the cavity of the host-cells cannot be completely solved until the fungus is isolated and its enzymes determined. It appears certain that the hyphae contain an enzyme which dissolves cellulose and another which dissolves the middle lamella, but less certain that they are able to react on suberized walls. The frequent occurrence of infecting hyphae between the radial walls of the lightly suberized epidermal layer and their passage from thence into the cavity, the absence or delay of infection of the sub-epidermal suberized layer, suggest that the hyphae have some difficulty in piercing these walls.

A point of importance which can here only be touched upon, and which awaits experimental work for its solution, is the exchange of nutritive materials between the endophyte and the host; on this partly rests the decision for symbiosis or parasitism (in so far as the relationship can be determined by this consideration of a limited part of the life-history). There is no doubt that the fungus benefits by the mucilage found on the surface of the tubercle. The concentration or constitution of this medium alters the form of its growth, and it is a vigorous and rampant form when compared with that of the hyphae on the main roots. Moreover, the production of the setae seems to indicate that it is at this stage that the fungus reaches the climax of its morphological development. As an ectotroph it is undoubtedly successful. On the other hand, as an endotroph it encounters vigorous resistance on the part of the host cells in the tubercle. There is never an abundant growth of intracellular hyphae, only a few threads are to be distinguished entire and the rest in a more or less digested condition. In the circumstances, it is difficult to see in what way the endophyte can gain food at the expense of the host. It seems more probable that the host cells gain something from the digestion of the endophyte, for the occurrence of exogenous branching in the presence of an endodermis indicates a supply of available food material in the peripheral regions, and this could only be supplied through the fungus or its digested remains. But even so, growth is very slow when compared with that of a normal root, and it would appear that the disturbance to the metabolism is greater than the nutritive gain. Moreover, the tubercles are impermanent as compared with lateral roots, and in many cases are shed after a year's growth, so that the main body of the plant gains nothing, unless, indeed, there is nitrogen fixation, and the discarded tubercles enrich the soil as do the similar structures in the Leguminosae.

It is not possible completely to state the relation between host and endophyte from the observational data described above, but in so far as it is revealed in the form and growth of the tubercles, I would describe the relationship not as symbiosis, if that implies the conferring of a permanent, mutual benefit, but rather as a state of balanced parasitism, in which, if there is any profit to either partner, it is alternating and impermanent.

SUMMARY.

1. The root-tubercles of *Arbutus Unedo* are shown to be the arrested secondary and successive laterals of the season's growth.

2. The arrest of growth is due to the invasion of a fungus, first as a rampant ectotroph and subsequently as an endotroph in the peripheral cells.

3. The invasion of the fungus is limited by the digestive action of the host-cells of the tubercle, which may be regarded as phagocytes, in the same sense as that in which Bernard applied this term to certain of the root-cells in the orchids.

4. As far as can be gathered from observational data, the interchange of food materials between host and endophyte is very slight and does not afford permanent mutual benefit.

5. The relationship is regarded as one of balanced parasitism, and the action of the tubercles is effective in conferring a degree of immunity on the root system as a whole.

In conclusion, I wish to offer my thanks to Dr. Rayner, both for the suggestion of this problem and for valuable advice and comment.

BEDFORD COLLEGE.

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Campbellia aurantiaca, Wight, and Christisonia albida, Thwaites.

BY

T. PETCH, B.A., B.Sc.,

Botanist and Mycologist, Ceylon.

With twenty Figures in the Text.

IN 1910 I gathered a *Campbellia* (Orobanchaceae) at Hakgala, Ceylon, at an elevation of 6,000 ft. It was parasitic on *Strobilanthes*, and in places it literally carpeted the ground. The *Strobilanthes* was then in flower. No identification of the *Campbellia* could be obtained.

Towards the end of 1922 the *Strobilanthes* on this area flowered again, and on visiting the spot in February 1923 the *Campbellia* was again collected. It was not as abundant as on the previous occasion, though very many groups of it were seen. Unfortunately the flowering season was nearly over, and only a few flowers were obtained. On a later visit, in September, both the *Strobilanthes* and the parasite were found to be dead.

The species of *Strobilanthes* on this particular area are *S. sexennis*, Nees, and *S. pulcherrimus*, And. The *Campbellia* was found only on the former. This is the largest species of the *Strobilanthes* of Ceylon, a small tree, 10–12 ft. high, with a stem about 4 in. in diameter. It was named *sexennis* because it was supposed to bloom once in six years, but it has been known for a long time that that estimate was incorrect. The area in question is traversed by the path from the Hakgala Botanic Garden to the top of the peak, and the condition of the *Strobilanthes* has been noted from time to time since 1910. It is quite certain that it did not flower between 1910 and 1922–3. The period between successive flowerings of *S. sexennis* at Hakgala was consequently twelve years in this instance. After flowering, the plants die.

It would have been of interest to have had continuous observations on the occurrence of the *Campbellia* during the period 1910–22, but such could only have been made by a resident in the district. There would appear to be some probability that these root parasites of *Strobilanthes* only come

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into flower when the *Strobilanthes* flowers and dies. On the other hand, the fact that this particular species has not been observed at other times might possibly be attributed to the lack of any inducement to the average botanist to push his way through the dense masses which the larger *Strobilanthes* form when they are in full foliage. It is only when they begin to flower, and the foliage is partly cast off, that one can see a way through the trees, and only when the plants are dead and their decaying stems can be pushed aside that one can penetrate between them without cutting a path. In the present case, however, a path runs through this *Strobilanthes* area, and if the parasite flowered regularly it would be expected that some of the numerous visitors to the top of the rock would have observed it. The path is only a narrow one, and well shaded. Any possible greater exposure to light along the sides of the path cannot have affected the distribution of the parasite, as it grows quite well there at the time the *Strobilanthes* flowers.

If the foregoing supposition is correct, the opportunity of finding in flower one of these Orobanchaceae which is parasitic on *Strobilanthes* comes only when the *Strobilanthes* flowers and dies. And if the parasitism of the *Campbellia* or *Christisonia* is limited to one species of *Strobilanthes* (as it is in this case as regards *S. sexennis* and *S. pulcherrimus*) the chance becomes very rare. In the case of *S. sexennis*, the parasite would be in flower only once in twelve years in any one station. Different areas of *S. sexennis*, however, do not all flower and die in the same year. At Hakgala there are at least two areas of *S. sexennis* which flower and die at different times. One of these flowered in 1905–6, and again in 1917–18.

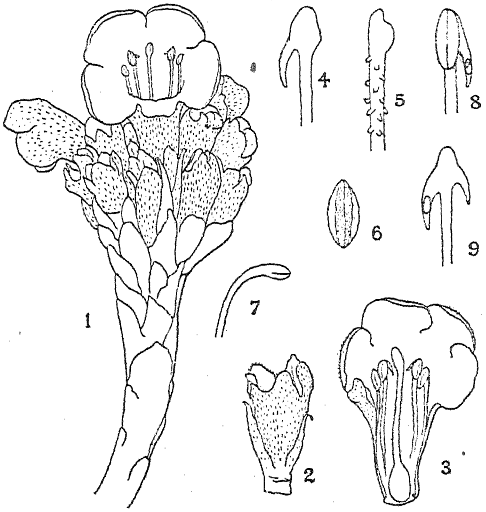
Strobilanthes sexennis also flowered on Pedrotalagalla in 1922–3. In September of the latter year the *Campbellia* was found in this locality, but the plants, like the *Strobilanthes*, were completely dead.

On examining the paintings of Orobanchaceae in the Peradeniya herbarium, it was found that there was one of this species. It shows a plant agreeing in all details with the recent collection, entirely pale yellowish white, or white with a yellow tinge within the corolla. This is dated August 1869. Further details, however, were added later, from plants collected on Pedrotalagalla in August 1884; and the whole was labelled *Campbellia cytinoides*, Wight. These later details do really belong to *Campbellia cytinoides*, but in the figure of 1869 the anthers and calyx are quite different. The specimens of August 1869 are included in the duplicates of C.P. No. 3929, *Christisonia albida*, in 'Herb. Peradeniya'.

Our plant was subsequently collected by Trimen in 1881, and referred by him to *Campbellia cytinoides*. The herbarium sheet bears his inscription, 'Hakgala Hill, near summit, 1881, bright yellow'. It may be noted that this is twelve years after the Thwaites's collection, and fits in with the flowering of *Strobilanthes sexennis* in 1905–6, the area at the summit.

The stem is short and stout, projecting only about an inch above the soil. It bears a dense head of flowers, which are borne racemosely on short stout pedicels (Fig. 1). The flowering stems arise close together, so that the heads form a compact flat cushion, often a foot or more in diameter, and scarcely elevated above the surrounding soil. As a rule, the whole plant—stems, bracts, calyces, and corollas—is yellow, but it may be so pale as to be almost white, or the corolla may be paler than the other parts.

The bracts, which are scattered on the subterranean part of the stem but densely imbricated above, are broadly ovate or oblong, usually with an obtuse apex. The pedicels are short, very stout, and bear two oval, boat-shaped, subacute bracteoles, which usually arise at the middle of the pedicel and extend over the base of the calyx (Fig. 2). The bracts and bracteoles are sparsely covered with long hairs. The calyx is tubular, pointed in bud, about 2 cm. high, obscurely five-angled, with five short triangular lobes, which, as a rule, soon become damaged, lacerated, and blackened; it is clothed with long viscid hairs at first, but may become more or less glabrous and scabrid.



FIGS. 1–9. *Campbellia aurantiaca.* 1. Flowering shoot. Natural size. 2. Calyx and bracteoles. Natural size. 3. Corolla, opened to show stamens and style. Natural size. 4. Inner stamen, viewed from the back. ×4. 5. Outer stamen, from the back. ×4. 6. Open anther. ×4. 7. Style. ×3. 8. Abnormal stamen. ×4. 9. Abnormal stamen. ×4. Figs. 1–3 copied from the painting of August 1869.

The corolla either does not greatly exceed the calyx in length, or it may be one and a half times as long. It is tubular, with five short, rounded, incurved lobes, the lowest lobe smaller than the others. The flower seldom expands widely; as a rule, the lobes remain incurved and the flower partly closed. The exterior is densely covered with long, lax, viscid hairs, which are up to 100 μ diameter at the base, closely septate, attenuated upwards to about 30 μ, and terminating in a flattened-globose glandular head, 50–60 μ diameter.

The stamens are didynamous, but the anthers all attain nearly the same level (Fig. 8). The filaments are flattened and adherent to the corolla for a considerable distance, the free parts being comparatively short. The two shorter filaments are usually glabrous, while the longer are papillose above, with short, cylindrical, rigid projections, 100 μ high, 80 μ diameter, which increase in length downwards until at the base of the filament they become hairs 450 μ long and 100 μ diameter.

The anthers are two-locular (one-celled in the older phraseology), oblong-oval, acute above. They dehisce down the middle, and open widely, the wall between the loculi remaining as an evident ridge down the centre (Fig. 6). The anthers are quite separate; they do not cohere, as is generally the case in *Christisonia*.

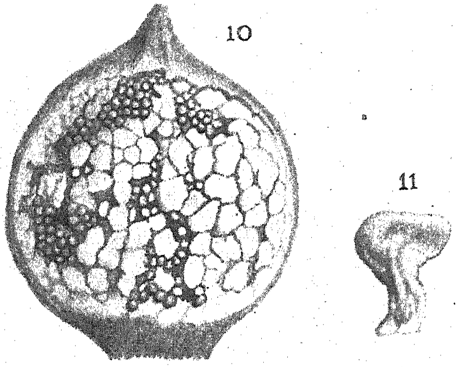
The shorter (inner) stamens bear a decurved pointed spur (Fig. 4), about as long as the anther, laterally. The longer stamens either have a similar but smaller spur, or they may bear merely a rounded, laterally flattened lobe without a basal point (Fig. 5). These spurs are prolongations of the connective, not barren, anther cells. Some variations of these are described below.

The style is glabrous, curved above, with the stigma projecting forward horizontally or slightly decurved. The stigma is clavate or ovoid, entire, with a narrow line, subtranslucent in the fresh specimens, along either side and round the tip (Fig. 7). The ovary is two-celled, with axile placentation (Fig. 14), the placentas, two in each loculus, arising from and above the centre of the septum (Fig. 12). But the septum is perforated by a narrow, vertical, median gap above the middle (see later description).

The fruit is globose, about 0·8 cm. diameter, crowned with the conical remains of the style. In the ripe fruit, the placentas occupy the greater part of the cavity and form four more or less kidney-shaped bodies, attached to the septum (Fig. 13). They bear seeds, &c., over the whole of their free surfaces, i.e. both on the outer surface and on the side next the septum.

When the thin wall of the fruit is scaled off, one finds a tessellated surface, either continuous, or interrupted here and there by groups of seeds (Fig. 10). The tessellated appearance is due to a series of bodies, usually turbinate in shape, with a short stalk and an expanded, flat, polygonal top, but sometimes simply dumb-bell-shaped (Fig. 11). These arise from the placentas, between the seeds, and the latter are situated in the cavities between them. Here and there, however, these bodies are absent, so that when the fruit wall is removed the ends of the seeds are immediately visible. These bodies are masses of parenchyma, and do not contain an embryo; their outer cell-walls are strongly thickened, so that they appear reticulated like the normal seeds.

These bodies do not appear to have been recorded by the describers of species of *Christisonia* or *Campbellia.* In Bentham and Hooker's 'Genera Plantarum', however, it is stated, with regard to *Campbellia*, that the seeds are very numerous, sometimes most or all ovoid or oblong-ovoid, with a lax, reticulated testa, sometimes some perfect, ovoid, with a striate testa, others abortive, usually longer than the perfect seeds, with a hyaline, reticulated, loose testa but without a nucleus. It is probable that the latter statement was based on an examination of the same species as that under consideration: the seed-coat appears striate under a low magnification, but is really reticulated.



FIGS. 10, 11. *Campbellia aurantiaca.* 10. The fruit, wall removed. × 5. 11. A parenchymatous body. × 15.

Variations from the normal flower appear to be not uncommon. In one flower, which had a four-lobed corolla, the following abnormalities of the stamens occurred: On the one side the filaments of the longer and the shorter stamens were connate, and both papillose; they became free towards the apex, and both anthers were spurred. On the other side the shorter stamen was represented by a short filament, with two spurs at the apex, one on either side, and a rudimentary anther cell on one spur (Fig. 9); while the longer stamen had a papillose filament, a normal anther, and a rudimentary anther cell on the spur (Fig. 8). Again, on examining Trimen's specimens collected in 1881, one of the shorter stamens of the flower examined was found to have an anther cell on the spur, in addition to the normal anther.

A large aphid was found on and in the flowers.

The particular features, or rather the combination of them, which makes the classification of this plant a matter of doubt, are (1) the bilocular ovary, with axile placentation; (2) the presence of parenchymatous bodies between the seeds; (3) the spurred stamens; (4) the longitudinal dehiscence of the anthers; (5) the simple stigma. On its bilocular ovary and simple stigma it is *Campbellia* (of Bentham); on the spurred stamens it is *Christisonia*; on the mode of dehiscence of the anthers it is neither (according to the generic descriptions).

In *Christisonia* the spur, according to the descriptions, is not an outgrowth of the connective or filament, but one half of the anther which is always barren. That is true of *Ch. bicolor*. But Worsdell found that in *Ch. subacaulis* the spur was an outgrowth of the connective, and an examination of the specimens of that species in 'Herb. Peradeniya' confirms that.

As regards the dehiscence of the anthers, the descriptions state that in both *Christisonia* and *Campbellia* the anthers open by a terminal pore. That is true of *Campbellia cytinoides*, *Christisonia bicolor*, *Ch. Thwaitesii*, and *Ch. tricolor*. But, according to the figures and specimens of *Ch. subacaulis* in 'Herb. Peradeniya', the dehiscence of the anthers in the latter species is longitudinal, and Wight's figure (Pl. 1423) shows the same.

In considering the descriptions of the older botanists, it has to be remembered that they described an anther from its appearance after dehiscence, and hence regarded a normal quadrilocular anther as two-celled. The bilocular half anther which occurs in *Campbellia* and *Christisonia* was consequently one-celled in their phraseology.

Wight instituted the genus *Campbellia* for the reception of two South Indian species of Orobanchaceae, *Campbellia aurantiaca*, Wight, and *Campbellia cytinoides*, (Reuter) Wight. The generic characters of chief importance are: (1) stamens didynamous, anthers one-celled, opening by a pore at the apex; (2) style simple, stigma capitate; (3) ovary spuriously two-celled at the base, one-celled at the apex, placentation parietal; (4) calyx bibracteolate. He considered that it was distinct from *Christisonia* on account of its bracteolate flowers and one-celled anthers. With regard to the latter point, Wight considered the anther of *Christisonia* two-celled, counting both the functional half anther and the barren half which forms the spur in some species of *Christisonia*. Gardner called the same anther one-celled, not counting the spur.

Campbellia aurantiaca, Wight, had sessile stipulate flowers, with lanceolate bracteoles; a five-lobed corolla scarcely exceeding the calyx, pubescent within; stamens scarcely didynamous with pilose filaments; and a pilose style with a clavate stigma. Wight noted that, 'As seen growing, this is a peculiar looking plant, the deep orange-coloured tops only appearing above the ground. This colour is derived from the bracts and calyx, the corolla being pale yellow, nearly white.' One of the anthers in the dissected flowers is represented two-celled; this is, I believe, an error of the artist, as

I have, since the plate was printed, examined recent specimens, and find them one-celled, as shown in the detached figures of the anthers, drawn at the same time as the rest of the picture.'

Campbellia cytinoides, (Reuter) Wight, was like the preceding species in general appearance, but 'certainly distinct'. It had lanceolate bracteoles, glabrous filaments, a one-celled deflexed anther, and a style hooked at the apex, with a clavate stigma.

Confusion began with the original publication. Wight's figures of *Campbellia cytinoides* show a one-celled ovary, with parietal placentation, but anthers which dehisce longitudinally, contrary to his generic description. His figure of *Campbellia aurantiaca* shows a corolla pubescent on both surfaces, and anthers opening by a pore and two-celled (i. e. furnished with a spur), while two of the three figures of the ovary show a completely bilocular ovary, with axile placentation.

Wight had previously sent specimens of the second species to Reuter, who had described them, together with specimens collected in South India by Perrottet, as *Phelipaea* (?) *cytinoides*. Reuter's description does not afford much assistance; he stated, with a query, that the anthers opened by a pore. Wight also sent specimens to Gardner, who included it in his genus *Christisonia*, and published a description under the name *Christisonia neilgherrica*. Gardner's genus *Christisonia* had a one-celled ovary, with parietal placentation, spurred anthers opening by a pore at the apex, and a bilobed stigma. In his description of Wight's specimens, he stated that the ovary is one-celled, with parietal placentation, that the anthers cohere with one another, that they open by an apical pore and have a conical, obtuse, deflexed spur at the base, and that the stigma is two-lobed, the upper lobe being abortive. The latter view is somewhat fanciful, and the remainder of the description suggests that Gardner had a mixture of two species. The anthers of *Campbellia cytinoides* do not cohere, they are not spurred, and the placentation is not parietal.

Bentham, in 'Genera Plantarum', retained *Christisonia* in Orobanchaceae, but transferred *Campbellia* to Scrophulariaceae. According to him, *Christisonia* had a two-lobed stigma; a one-celled ovary with parietal placentation; and anthers of which one loculus was fertile, while the other was barren and spur-like. *Campbellia*, on the other hand, had a two-celled ovary (? or shortly one-celled at the apex) with two placentas in each loculus; stigma capitate, oblong, or peltate; and anthers as in *Christisonia* or with the barren loculus wanting.

Hooker, in 'Flora of British India', united *Campbellia* with *Christisonia* in the Orobanchaceae, and included Wight's two species under Gardner's name, *Christisonia neilgherrica*, though he queried the inclusion of *Campbellia aurantiaca*.

Trimen, in 'Journal of Botany', xxiii, p. 240, wrote: '*C. neilgherrica* is rightly made synonymous with *Campbellia cytinoides*, Wight, in the "Fl. Brit. India". . . . It is not infrequent in the high mountainous forests here, growing on the roots of *Strobilanthes*, and I have had several opportunities of examining fresh specimens. I am inclined to think Wight's genus a good one, and its reunion with *Christisonia* by Sir J. Hooker to be uncalled for. The structure of the anthers is very different in *Campbellia*; they are strictly one-celled, open by a terminal pore, and are quite free from one another and without spurs: the style, too, is different, being hooked, ovoid, and quite entire. The genera are, however, closely allied, and their station away from one another in different natural orders, as in the "Gen. Plant.", is clearly an extreme in the opposite direction. The structure of the ovary and placentas is quite the same in both genera, truly one-celled in all I have examined, except occasionally near the base.' Accordingly, in the 'Handbook of the Flora of Ceylon', Trimen retained the genus *Campbellia*, distinguishing it from *Christisonia* by its clavate stigma and anthers without spurs. It must, however, be noted that Trimen based his opinion on *Campbellia cytinoides*, which, from the order of publication is not the type species of the genus *Campbellia*.

Campbellia cytinoides is a fairly well-known plant. It has a simple, clavate stigma, and deflexed anthers which open by a pore at the apex. The apex of the filament is slightly thickened, but it is not spurred.

It follows from the foregoing that Wight's plate of *Campbellia cytinoides* (No. 1425) is incorrect,[1] since it shows anthers which dehisce longitudinally. If, however, we interchange the anthers of Pl. 1425, Fig. 4, with those of Pl. 1424, Fig. 3, the former becomes correct for *Campbellia cytinoides*, while the latter is then a good representation of the unnamed Ceylon species from Hakgala. It seems most probable that in this particular the draughtsman confused the two species; that could easily happen, since, as stated by Wight, they resemble one another in general appearance.

The Ceylon species agrees with the figures of *Campbellia aurantiaca* in the shape of the corolla, calyx, and bracteoles; the shape of the stigma, with its lateral line; the spurred anthers, as depicted on the dissected corolla; the pilose filaments; and the bilocular ovary, as shown in Figs. 5 and 7. The points in which it does not agree (after the interchange suggested) are the pilose style and the pilose inner surface of the corolla. With regard to the corolla, the Ceylon species is hairy externally, but glabrous internally, except at the base of the filaments. Wight stated that the corolla of *Campbellia aurantiaca* is pubescent within, but his artist figured it pubescent on both surfaces. It may be noted that Pl. 1425, Fig. 4, shows glabrous filaments; these are normal for the shorter stamens of the Ceylon species.

It would appear to be a fair conclusion, therefore, that the Ceylon species in question is *Campbellia aurantiaca*, Wight. It resembles *Campbellia cytinoides* so closely in general appearance that Trimen assigned his gathering of it to the latter species.

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[1] Numerous errors in Wight's plates were noted by Wight himself.

Bentham probably examined *Campbellia aurantiaca* for the characters of the genus *Campbellia*, as he described the ovary as two-celled, and noted the peculiar parenchymatous bodies which accompany the seeds. On the other hand, Hooker probably examined *Campbellia cytinoides*, as he united the genus to *Christisonia*.

The separation of the species of *Christisonia* and *Campbellia* into different genera will naturally depend on the relative importance attached to the different characters. We have, in the different species, anthers which open by a pore and anthers with longitudinal dehiscence; styles simple, clavate, or expanded and lobed; anthers with a spur formed by a barren anther cell, others with a spur which is an outgrowth of the connective, and others which are not spurred; a one-celled ovary with parietal placentation, or a two-celled ovary with axile placentation; and these characters occur in various combinations.

Wight's differentiation by means of the bracteoles and anthers does not lead to a natural separation. *Christisonia bicolor* has paired bracteoles, a one-celled ovary, a lobed stigma, and anthers which open by a pore and are furnished with a spur consisting of a barren anther cell. *Campbellia cytinoides* has paired bracteoles, a one-celled ovary, a simple stigma, and anthers which open by a pore and are not spurred. *Campbellia aurantiaca* has paired bracteoles, a two-celled ovary, a simple stigma, and anthers which dehisce longitudinally and have a spur which is an outgrowth of the connective.

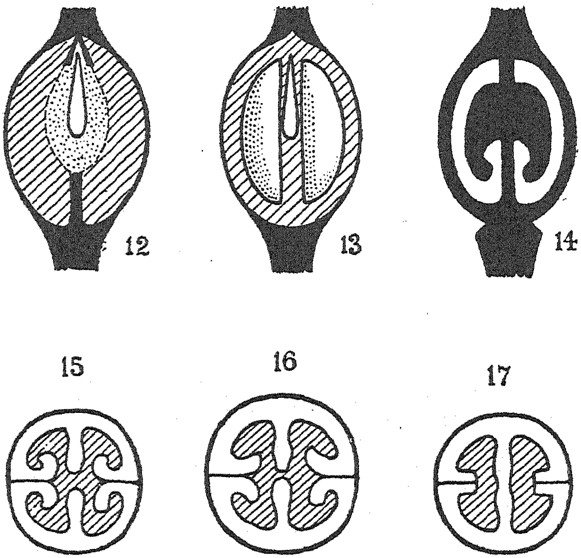
From the order of publication, *Campbellia aurantiaca* is the type species of the genus *Campbellia*. But Wight's generic description is based on *Campbellia cytinoides*, as he described the anthers as opening by a pore, and the ovary as spuriously two-celled at the base, one-celled at the apex. Bentham described the ovary of *Campbellia* as two-celled, or (with a query) shortly one-celled at the apex; this covers both *Campbellia aurantiaca* and *Campbellia cytinoides*.

In *Campbellia aurantiaca* the ovary is two-celled, the placentation being axile, with two placentas in each cell.

In *Campbellia cytinoides*, Wight found the ovary spuriously two-celled at the base, one-celled at the apex, and considered the placentation parietal. Gardner stated that the ovary was one-celled and the placentation parietal. Trimen stated that the structure of the ovary and the placentas was the same in both *Campbellia* and *Christisonia*, the ovary being truly one-celled except occasionally near the base. Worsdell, however, wrote:

'As stated in my former paper, the ovary of *Christisonia* is unilocular, with two bipartite placentas. In one of the species (*C. neilgherrica*, Gard.), however, there is a slight modification, the lower portion of the ovary being bilocular owing to the fact that the placentas from opposite sides meet and fuse in the centre, whereby the ovules acquire an axile placentation ; in the upper part of the ovary the placentas again separate, and the ovary becomes unilocular.'

Christisonia neilgherrica, Gard., is *Campbellia cytinoides*, Wight. Consequently Worsdell observed the same structure as noted by Wight, Bentham, and Trimen. But the interpretation is not quite correct. A previous knowledge of *Christisonia* appears to have misled the majority of those who have examined *Campbellia*.



FIGS. 12–17. *Campbellia aurantiaca*. 12. Longitudinal median section of the fruit, showing the septum (obliquely lined), the area of attachment of the placentas (dotted), and the median perforation. ×3. 13. Fruit, wall and seeds removed, showing the two placentas of one loculus. ×3. 14. Longitudinal section of ovary, perpendicular to the septum, slightly to one side of the median plane. ×3. 15. Transverse section of ovary in lower half, placentas obliquely lined. ×3. 16. Transverse section of ovary, about the middle. ×3. 17. Transverse section of ovary in upper half. ×3.

In *Christisonia* a placenta arises along each side of the ovary, projecting inwards as a vertical plate, which, near the middle of the ovary, divides longitudinally into two lateral lobes which curve outwards. In a cross-section of the ovary, therefore, one sees two T-shaped placentas, the stalk of the T arising from the ovary wall, and the two cross-bars parallel to one another and almost in contact. The cross-bars, or arms, of the T are, of course, not straight, but variously inrolled at their extremities. In the ripe capsules of *Christisonia Thwaitesii* the placentas arise from two ribs, one on each side, adnate to the wall of the ovary ; while in *Ch. tricolor* these ribs are stout rounded columns, which, when the thin wall of the capsule is broken away, curve upwards like a pair of horns from the apex of the pedicel.

In *Campbellia aurantiaca* the ovary is divided into two loculi by a vertical septum (Fig. 12). A thickened strand traverses this septum medially from the base of the capsule to the area of attachment of the placentas. At about half the height of the capsule, the bases of the two placentas in each loculus separate from one another, but remain attached to the septum, while the latter is perforated by a narrow slit between the upper halves of the placentas. From the apex of each placenta a stout strand runs obliquely upwards, these strands ultimately uniting with one another and to the apex of the ovary.

Thus, if the ovary of *Campbellia aurantiaca* is cut transversely about the centre, it appears bilocular, with axile placentation (Fig. 16). If, however, a transverse section is taken in the upper half, it appears unilocular, and the placentation parietal (Fig. 17). But the dissection of the ovary shows that the latter appearance is fictitious; the apparent 'stalks' of the placentas are parts of the septum, and the ovary appears unilocular because the septum is perforated along the median line. The placentation is axile and the capsule imperfectly bilocular.

In *Campbellia cytinoides* a septum is present in the lower half of the ovary. From the middle of this imperfect septum four placentas arise, two in each cell; and the median strand of the septum is continued upwards as a central column to the apex of the ovary, where it unites with the ovary wall. In the upper part of the ovary the placentas are attached to the central column. The upper part of the ovary is one-celled, but has an axile column. Consequently the structure of the ovary is essentially the same as in *Campbellia aurantiaca*, but the septum is lacking in the upper half on either side of the median line. The placentation is axile; its description as parietal is due to mistaking the septum for the stalk of the T-shaped placenta of *Christisonia*.

Bentham separated *Campbellia* from *Christisonia* on the structure of the ovary. In both *Campbellia aurantiaca* and *Campbellia cytinoides* the ovary may be regarded as two-celled, the septum being imperfect. In both the placentation is axile and the stigma simple. The two species differ in the structure of the anther. It would appear that Bentham's conclusion, that *Campbellia* should be included in the Scrophulariaceae, is correct, in spite of the general resemblance and similar habit to *Christisonia*.

From Wight's figures, *Christisonia calcarata*, Wight, and *Christisonia Lawii*, Wight, appear to have a two-celled ovary, and would consequently be included in *Campbellia*. But with regard to the former, Wight stated that 'it seems not improbable that some parts of the analysis may be found faulty, as they are difficult plants to dissect from dried specimens'; and

As regards *Christisonia albida*, we know that Thwaites was at Peradeniya in October 1866, and on October 6 he wrote to Ferguson, 'My collectors are still out, and I trust to see them back in a few days'. The evidence, therefore, indicates that Thwaites did not collect the *Christisonia albida* of 1866, but that it was brought in from Hakgala by his collectors.

Further, there is no evidence that the specimens were collected in the jungle on the Botanic Garden reserve. His collectors may have been collecting in the Hakgala district, and they may have obtained the specimens in the jungle behind the hill, or on the opposite side of the valley, both of which are still practically unworked ground.

Again, although the jungle borders the Botanic Garden, and many botanists have worked there, the area which they have investigated is very limited. There is only one path through the jungle reserve, viz. that from the Garden to the top of the rock. For a few years, from 1905, there was a path into the jungle for a short distance from the middle of the Garden, but it was soon overgrown. For the remainder of the jungle, one has to take advantage of temporary wood-cutters' paths or elephant tracks, or to scramble along the streams, exploring laterally by cutting a way through the undergrowth or pushing through the more open spaces. Botanists have usually confined themselves to the path to the peak, and even there no one collected *Campbellia aurantiaca* between 1881 and 1910, though it grows on both sides of the path over a distance of about 100 yards. That the Hakgala jungle reserve has not been thoroughly searched was demonstrated this year (1923) by the discovery of a new species of *Sciaphila* in it. This was found above the Garden, within a hundred yards of the boundary, and in the ravines on the patana to the south, its stations extending over a distance of a mile and a half. It is evidently generally distributed through the jungle, but the only locality visited in which it was not found was along the path to the peak. The latter traverses a part of the jungle which has a slightly different aspect and rainfall, and it may be that the *Sciaphila* does not occur in that area.

The first specimens which have been assigned to *Christisonia albida*, Thw. (C.P. 3929), were collected in October 1866. As already stated, the evidence indicates that these were gathered by Thwaites's collectors, and that he did not see them in the fresh state. There is no painting of that collection. In August–September 1869 the draughtsman made two paintings of Orobanchaceae at Hakgala. These are two different species, and both different from the specimens of 1866. But by some mistake the specimens of 1869 were placed in the herbarium under C.P. No. 3929, i. e. the same number as the specimens of 1866. It is difficult to understand how this happened, as the plants are so very different in general appearance. A possible solution is that the draughtsman had been sent up to Hakgala to obtain a drawing of the C.P. 3929 of 1866, and therefore put the plants of 1869 under that number. It would appear from this confusion that Thwaites did not personally collect the plants of 1869.

Examination of the specimens show that the plants collected in 1866 are *Christisonia bicolor*, Gardn., var. *spectabilis*, Trimen; those of August 1869 are *Campbellia aurantiaca*, Wight; those of September 1869 are *Christisonia Thwaitesii*, Trimen; and there is also under the same C.P. number a single specimen of *Christisonia bicolor*, Gardn. It almost appears from the herbarium specimens that C.P. 3929 was a general number for all specimens of Orobanchaceae collected at Hakgala.

The specimens of 1866 were mounted by Thwaites, but he did not label the sheet. It bears the C.P. number 3929, and the note 'Hakgala, October 1866', in his handwriting. Trimen added the name '*Christisonia albida* Thw. MSS.', and the note 'tota planta albida (Thwaites)'. I have not been able to discover whence Trimen obtained the latter note. His reference to Thwaites's notes may refer to a manuscript list of additional C.P. numbers, bound up with Thwaites's copy of his 'Enumeratio' in the Peradeniya library, in which occurs the entry '3929 *Christisonia albida* Thwaites MSS. Tota planta albida', but that list is not in Thwaites's handwriting.

The specimens of 1869 were left unmounted as duplicates of C.P. 3929, accompanied by a label in Thwaites's handwriting, 'C.P. 3929, *Christisonia* sp. Hakgala, October, 1866'. This is probably the Thwaites label of the original gathering, and it is to be observed that it does not bear the name *Christisonia albida* nor any note of the colour. In 1901, when a laboratory was opened at Hakgala, a herbarium of Hakgala and up-country plants was instituted there, duplicates being transferred from Peradeniya; and *Christisonia albida* was represented by a copy of the painting of September 1869, with a sheet of specimens made up from the duplicates of C.P. 3929. Thus, while *Christisonia albida* in 'Herb. Peradeniya' was *Christisonia bicolor*, var. *spectabilis*, *Christisonia albida* in 'Herb. Hakgala' was a mixture of *Campbellia aurantiaca* and *Christisonia Thwaitesii*, with a painting of the latter.

Bentham had specimens of *Christisonia albida* from Thwaites, and in 'Genera Plantarum' he transferred it to *Campbellia*, because the ovary was completely two-celled. From that, and his description of the parenchymatous bodies which accompany the seeds, it is evident that Bentham had specimens of the collection of August 1869, viz. *Campbellia aurantiaca*.

In 'Flora of British India' Hooker gave the first description of *Christisonia albida* as follows (I have italicized the more striking differences between this description and Trimen's):

'*C. albida* Thw. MSS.; *sparsely pubescent*, stem very short, scales very obtuse, flowers crowded, *very shortly pedicelled*, calyx 2-bracteolate, *lobes rounded*, filaments very short, anthers all unequally 2-celled, cells oblong parallel.

'Stem 1–2 in. from an elongate warted rhizome like that of *C. bicolor*, very stout. Scales ¼ inch, obovate, tip rounded. Flowers crowded, *much smaller than in any of the other species.* Calyx ⅓ *inch long*, *pubescent*, lobes very short, erose. Corolla 1 in., pubescent externally. Anther cells acute below. Ovary completely two-celled, stigma very small, clavate.'

The above description evidently refers entirely to the specimens of August 1869, viz. to *Campbellia aurantiaca*, Wight. Hooker's material was probably the same as Bentham's.

In a foot-note in the 'Journal of Botany', xxiii, p. 241, Trimen wrote, '*Christisonia albida* Thw., referred to Campbellia in Gen. Plant, ii, p. 967, is rightly put back again into Christisonia in the Fl. Brit. India.' But he, naturally, had examined the earliest specimens of C.P. 3929, the only mounted sheet in 'Herb. Peradeniya', and so had based his opinion on the specimens of 1866, viz. *Christisonia bicolor*, var. *spectabilis.*

In the 'Handbook of the Flora of Ceylon', Trimen gave a fuller, but not a complete, description:

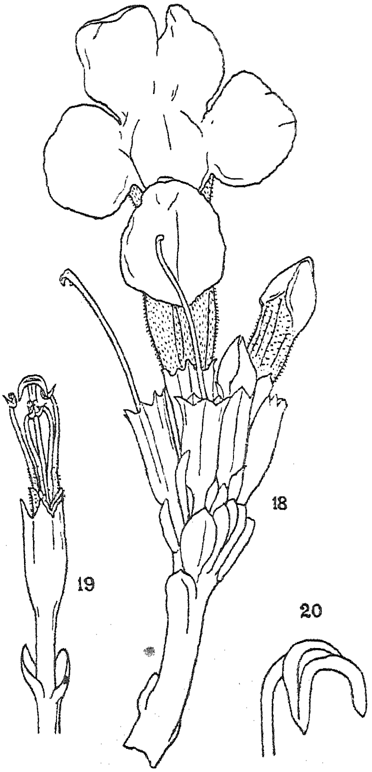
'Scape short, scales ovate *glabrous*; *flowers large*, crowded, *pedicel* ½ *in.*, with two bractlets some distance below flower; calyx ¾ *in.*, *glabrous*, segments five, very small, *linear*; corolla tube 1½ inch or more, pubescent outside, limb 1½ in. diameter, lobes broad truncate.

'Upper montane zone; very rare. Hakgala, 1866 (Thwaites). Fl. October; white.

'The whole plant is white, according to Thwaites's notes. I have not met with it, and have only the C.P. specimens. Bentham referred it to Campbellia in Gen. Pl. (l. c.). The ovary is said in Fl. Brit. Ind. to be completely two-celled.'

This description is evidently based on the specimens of 1866, viz. *Christisonia bicolor*, var. *spectabilis.* The particulars given by Willis are taken from this description. As Trimen did not describe the stamens or stigma, it would appear that he did not dissect a flower. Had he done so, he would no doubt have discovered that the specimens were *Christisonia bicolor*, var. *spectabilis.*

The specimens of 1866 have a stout rhizome, which lacks the numerous thorny projections found on the thinner rhizome of *Christisonia bicolor*. The flowers are grouped together at the top of the scape, but owing to the length of the pedicels they are not crowded. The pedicels are 1–2·5 cm. long, with a pair of bracteoles, either below the middle or at the base. Trimen stated that the bracteoles were 'some distance below the flower', not 'below the flower', as quoted by Willis. The calyx is narrow, tubular, slightly expanded above, 1·5–2 cm. high, glabrous, with five teeth varying from triangular to almost linear. The corolla in the dried specimens is up to 7 cm. high, with a limb up to 4 cm. diameter, hairy externally; the tube is very narrow below, and expands upwards to about double the diameter. The anthers are spurred, the spur being a barren anther cell; the filaments are naked or slightly pubescent above, and pubescent with long hairs at the base. The stigma is lunate, and about 5 mm. from side to side. In all these points the specimens agree completely with *Christisonia bicolor*, var. *spectabilis* (Figs. 18–20).



FIGS. 18–20. *Christisonia spectabilis.* 18. Flowering shoot. × $\frac{2}{3}$. 19. Calyx, stamens, and style. × $\frac{2}{3}$. 20. Stigma, enlarged.

There would seem to be very little doubt which collection Thwaites intended to be *Christisonia albida.* The evidence indicates that he did not see any of the plants in a fresh state, and that, in consequence, any note of the colour when fresh must have been based on the statements of his collectors, or on the paintings made from the fresh specimens by the draughtsman. There is no painting of the collection of 1866, and that species, *Christisonia bicolor*, var. *spectabilis*, has normally a pink calyx, and a corolla with a white limb and pale yellow tube. On the other hand, the colour of the painting of *Campbellia aurantiaca*, made in August 1869, could undoubtedly be styled *albida*, and Thwaites sent specimens of that gathering to Kew. It would appear that *Christisonia albida*, Thw., applies to the specimens of August 1869, and that the name is consequently a synonym of *Campbellia aurantiaca*, Wight.

In any case, the alleged disappearance of *Christisonia albida* is due to the fact that C.P. 3929 was a mixture. Hooker's description of *Christisonia albida* does not refer to the same plant as Trimen's. Botanists relying on Trimen's description and the mounted specimens in 'Herb. Peradeniya' would have to search for a *Christisonia* with larger flowers than any other Ceylon species, the whole plant white in colour, and, as far as is yet known, no such plant has ever existed.

Christisonia bicolor, var. *spectabilis*, differs from *Christisonia bicolor* in its rhizome, its narrower calyx and corolla tube, its glabrous calyx, and its lunate stigma. *Christisonia bicolor* has a more or less reniform stigma, but that of var. *spectabilis* is laterally elongated, and decurved at the ends, resembling to some extent a croquet hoop (Fig. 20). In some of the herbarium specimens of the latter the stigma is a centimetre from side to side. This should be separated from *Christisonia bicolor* as *Christisonia spectabilis*, Thwaites.

SUMMARY.

1. *Campbellia aurantiaca*, Wight, is distinct from *Campbellia cytinoides*, Wight.

2. The genus *Campbellia* should be included in the Scrophulariaceae, where it was placed by Bentham.

3. *Christisonia albida*, Thwaites, so far as relates to the plants collected in 1866, is *Christisonia bicolor*, var. *spectabilis*, but according to the plants of August 1869, on which the first published description was based, it is *Campbellia aurantiaca*.

4. It is most probable that Thwaites regarded the plants of August 1869 as his *Christisonia albida*; in that case it has been collected again in 1881, 1910, and 1923, and occurs in South India.

5. *Christisonia bicolor*, var. *spectabilis*, should be considered a distinct species, *Christisonia spectabilis*, Thw., in Herb. This species is known from two widely separated localities in Ceylon.

6. A third species included in C.P. 3929, viz. *Christisonia Thwaitesii*, Trimen, is known from two localities in Ceylon.

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Some Observations on the Nitrate-reducing Properties of Plants.

BY

V. L. ANDERSON.

From the Botanical Department of University College, London.

IT would appear to be generally accepted that the ordinary green plant obtains its supply of nitrogen in the form of nitrate which is absorbed from the soil; that, since nitrate is a relatively inert substance, it is reduced to nitrite prior to its elaboration into a food substance; and that the nitrite must be fixed almost as soon as it is formed, since it is a very reactive substance and has marked toxic properties.[1] If these acceptances be true, amongst the problems immediately arising are the following: to identify the agent responsible for the reduction of the nitrate, and to determine its distribution in plants generally; to see whether nitrite is as rare in plants as is often stated; to find the mechanism for the disappearance of nitrite; and to ascertain the fate of the nitrite.

The observations of Haas and Hill[2] confirm and extend the work of earlier authors on the presence of a nitrate-reducing agent in milk; they call it atite, and show the presence in the same medium of a nitrite-oxidizing mechanism which reconverts the nitrite into nitrate. The observations recorded in the present preliminary communication are an outcome of the work of these two authors.

THE OCCURRENCE OF NITRATE IN PLANTS.

Brief reference must be made to the presence of nitrate in the plant, and that such is the case is shown by a mass of evidence which need not be considered here.[3, 4] In the present instance the presence of nitrate has

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[1] But see Czapek: Biochemie der Pflanze, i. 191.

[2] Haas and Hill: Biochem. Journ., xvii. 671.

[3] Klein: Beihefte Bot. Cent., xxx. 141.

[4] Molisch: Mikrochemie der Pflanze. Zweite Auflage, 91.

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been demonstrated by means of the diphenylamine reaction in the expressed sap of the shoots of the following plants:

Aegopodium Podagraria
Aesculus Hippocastanum
Atropa Belladonna
Avena sativa
Brassica campestris
„ *oleracea*
Clematis Vitalba
Grasses (various)
Helianthus tuberosum
Isatis tinctoria
Lactuca sativa
Lupinus, sp.
Mercurialis perennis
Nasturtium officinale
Physalis peruviana
„ *Alkekengii*
Raphanus sativus (root)
Reseda alba
„ *lutea*
Rubus Idaeus
Rumex acetosa
„ *conglomeratus*
„ *crispus*
Sambucus nigra
Scopalia lurida
Senecio vulgaris
Silene maritima
Solanum Dulcamara
„ *Lycopersicum*
„ *nigrum*
„ *tuberosum*
Sonchus arvensis
Spinacia oleracea
Suaeda fruticosa
Triticum vulgare
Tussilago Farfara
Ulva lactuca
Urtica dioica
Withiana somnifera

Also in traces in the seeds of *Brassica nigra, Cannabis sativa*, and *Lepidium sativum.*

That the amount of nitrate present in the plant may vary considerably during the life-cycle has been shown by Berthelot[1] and has been confirmed in several instances in the present work, e. g. *Mercurialis perennis* gave positive nitrate reactions in October and negative in June, and the leaves of *Lupinus* sp. were found to be much richer in nitrate in October than in June; in each case the material was taken from the same locality. That a daily fluctuation of nitrate content occurs in some plants has been shown in the case of *Solanum Dulcamara*, where the amount of nitrate present in the early morning was considerably less than that found in the late evening.

Berthelot[1] has also shown that the distribution of nitrate may be localized in the plant, an aspect more recently studied by Klein,[2] and in the present instance it was observed that, although nitrate was present in the leafy shoots of *Physalis Alkekengii*, *Solanum Dulcamara*, and *S. Lycopersicum*, no trace of it could be shown to exist in the berries, even when these were in an early stage of development. It was also found that although nitrate was present in the etiolated 'heart' of the cabbage, it could not be demonstrated in the sap from the outer green leaves, an observation which was paralleled by results from experiments on the seedlings of *Triticum* and *Avena*, where the etiolated material gave slightly stronger reactions than that grown under more normal conditions.

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[1] Berthelot: Compt. Rend., 1884.

[2] Klein, op. cit.

In certain instances it was found that plants from one locality gave negative results, when tested for nitrates, whilst individuals of the same species from other localities gave positive results. Thus *Suaeda fruticosa* grown on a shingle beach (from which soluble salts are readily leached) under conditions of maximum insolation gave negative results, whilst the same species grown in garden soil and subjected to a certain amount of shade gave a strong positive reaction for nitrate. Similarly, *Sambucus nigra* from an exposed hedge-bank on a poor soil gave an almost negative reaction, while material from another locality in a wooded valley was very rich in nitrate; and *Rumex conglomeratus* growing on an old manure heap gave much stronger reactions than the same species from a hedgerow. It would, therefore, appear that the presence of nitrate in the plant in amounts detectable by the agent employed depends to a considerable degree on the richness of the soil and the anabolic intensity. Thus the plant growing in a highly manured soil will give a strong positive reaction for nitrate, probably because it absorbs more than it can elaborate in a given time; whilst, on the other hand, a plant grown under conditions highly favourable for nitrogen assimilation, but in a poor soil, will give negative reactions for nitrate simply because the nitrate is elaborated at a rate equal to the rate of supply. If this generalization be accepted it would throw light upon some earlier and contradictory observations; thus among the plants mentioned by Klein as being very poor in nitrate content, there are many whose usual habitats are poor in soluble food substances, and whereas Molisch[1] finds nitrates to be generally absent in trees, Berthelot[2] cites them as occurring in such types as are usually well manured, e.g. *Prunus domestica* and *Pyrus communis*.

THE OCCURRENCE OF A NITRATE-REDUCING MECHANISM IN PLANTS.

Kastle and Elvove[3] were the first to demonstrate the power of the expressed sap of potato tubers to effect the reduction of sodium nitrate to nitrite in the presence of acetaldehyde and other accelerators.

Throughout the present work the method of procedure in detecting such a reducing mechanism was as follows:

To 10 c.c. of the expressed sap was added ten drops of 4 per cent.

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[1] Molisch, op. cit.

[2] Berthelot, op. cit.

[3] Kastle and Elvove: Amer. Chem. Journ., 1904, xxxi. 606.

sodium nitrate;[1] the liquid was then equally divided between three test-tubes, to one of which were added two drops of 10 per cent. acetaldehyde; the tubes were placed in a water-bath at 45° C.; the one without acetaldehyde was removed at the end of ten minutes, and the other two at the end of twenty minutes. After cooling, the extract was, if necessary, half saturated with ammonium sulphate, and filtered to remove chlorophyll and other substances, and was then tested for the presence of nitrite with Griess-Ilosvay reagent, some of the original extract being used for purposes of control.

The use of Griess-Ilosvay reagent for the detection of nitrite has certain disadvantages; it is acid in reaction, and, as Klein has pointed out, will react with any anthocyan present, producing a red colour which may be mistaken for the nitrite reaction. This may be obviated by the selection of parts free from anthocyan and by the use of control experiments. Another disadvantage lies in the fact that when there is relatively much nitrite present Griess-Ilosvay will give a pink colour which soon turns brown. This latter occurs only very rarely in plant extracts, and its effects in the present work may be discounted.

The work of earlier authors[2,3] on the presence of a nitrate-reducing mechanism in the potato tuber and in etiolated potato shoots has been confirmed. In addition, it has been found to obtain to a well-marked extent in the sap expressed from the green shoots of the potato and of *Solanum Dulcamara*, and also observed, though to a slight extent, in the expressed sap of the leafy shoots of the following plants:

Arum maculatum
Bryonia dioica
Clematis Vitalba
Dahlia, sp. (young tubers)
Helianthus annuus (etiolated seedlings)
Helianthus tuberosum
Linum usitatissimum (etiolated seedlings)
Lychnis alba
Medicago lupulina
Physalis Alkekengii
Physalis peruviana
Pisum sativum
Ranunculus bulbosus
Raphanus sativus
Rheum officinale (etiolated leaves)
Solanum crispum
„ *Lycopersicum*
Tamus communis
Trifolium pratense
Triticum vulgare
Viburnum Lantana.

In several of these plants the activity of the reducing mechanism has been shown to be variable, and this is markedly so in the case of *Solanum Dulcamara*; in material tested in October the reduction was scarcely discernible, while material tested in June gave good positive results. A number of experiments were carried out on June material, but so far as the reducing activity was concerned there was no variation shown, whether the material was obtained in the early morning or late evening or from varying habitats. This points to a well-marked seasonal variation; but in other plants the variations observed are not so easily explained; thus the etiolated seedlings of *Helianthus annuus* and *Linum usitatissimum*, which gave weak but decidedly positive results on one occasion, could not be confirmed after a lapse of some months, though the conditions of growth were apparently the same. It appears probable that another type of variation occurs in such cases, but further investigation will be required before this can be elucidated.

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[1] In cases where the extract was already rich in nitrate the amount added was modified slightly.

[2] Kastle and Elvove, op. cit.

[3] Bach: Biochem. Z., 1911, xxxiii. 282, and 1912, xxxviii. 154.

With respect to these observations it must be noted that, although Griess-Ilosvay is an extraordinarily delicate reagent, the absence of a positive reaction at the end of an experiment does not necessarily mean that a nitrate-reducing mechanism is absent. The expressed sap of the plant, which must of necessity be employed, may contain various substances which may enter into combination with the nitrite as soon as it is reduced from the nitrate, and a negative reaction with Griess-Ilosvay will therefore obtain even though a reduction may have occurred. Thus the expressed sap of *Rumex conglomeratus* contains tannins which immediately combine with added nitrite, and the same has been observed in several other plants (see below).

The work of Haas and Hill[1] indicates that the nitrate-reducing substance, atite, in milk is, although thermolabile, an oxidizable substance rather than an enzyme. The same conclusion has been arrived at with respect to the nitrate-reducing substance in the potato, which also is thermolabile, and is thrown out of solution by saturation with ammonium sulphate. The evidence is as follows: To 300 c.c. of the liquid expressed from potato tubers were added 3 c.c. of 10 per cent. acetaldehyde and 12 c.c. of 4 per cent. sodium nitrate. Since the potato extract contains an active oxidase, and also to prevent the oxidation of the nitrite, the air in the containing vessel was replaced by nitrogen, and the liquid was then placed in a thermostat at 45° C., where it was continually rotated; samples of 30 c.c. were removed periodically, precautions being taken to maintain the atmosphere of nitrogen in the vessel.

To the sample removed, an equal volume of saturated ammonium sulphate was added, and, after filtering, crystals of ammonium sulphate were added to effect complete saturation. On further filtration a clear liquid was obtained; to 30 c.c. of this were added 1 c.c. of metaphenylenediamine and 1 c.c. of sulphuric acid, while a standard solution containing

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[1] Haas and Hill, op. cit.

1 c.c. of 0·018 per cent. sodium nitrite in 30 c.c. of saturated ammonium sulphate and a proportional amount of acetaldehyde was similarly treated. These were allowed to stand for twenty minutes, by which time a golden brown colour had developed, and the standard was then diluted until its colour exactly matched that of the extract. The results obtained are expressed below:

Time.	*Relative amounts of nitrite.*
15 minutes	0·04 of standard
30 ,,	0·31 ,,
1 hour	0·36 ,,
1½ hours	0·38 ,,
2 ,,	0·55 ,,
3 ,,	0·62 ,,
4 ,,	0·87 ,,
5 ,,	0·87 ,,
6 ,,	0·87 ,,
7 ,,	0·87 ,,

From the fact that no increase in the amount of nitrite occurred after the fourth hour (nitrate being still present in abundance) it would appear that the nitrate-reducing substance, under the conditions of the experiment, is capable of reducing but a limited amount of nitrate. Although the substance may not be identical with the atite of milk it appears to be of generic relationship, and may be termed atite pending its isolation, before which a chemical examination is impossible.

In the expressed sap of *Solanum tuberosum*, *S. Dulcamara*, *Linum usitatissimum*, and certain other plants the action of the atite was undoubtedly accelerated by the presence of acetic aldehyde, but on the other hand in *Medicago lupulina* and several other plants the aldehyde had no apparent effect, while in *Tamus*, *Pisum*, and others it actually retarded the action of the atite. This appears to indicate that more than one mechanism may be involved in the initial reduction of nitrates in plants.

THE OCCURRENCE OF NITRITE IN THE PLANT.

The presence of an atite in the plant naturally leads to a search for nitrite. There is much conflicting evidence on the subject of the occurrence of this substance in the green plant. Molisch[1] considers that nitrites never occur, while Czapek[2] appears to credit various authors who have described their occurrence. Aso[3] finds nitrites in etiolated potato shoots, and Klein[4] in *Erythrina* and other plants, but the latter considers that the occurrence may often be due to the action of bacteria or fungi, and he cites the root nodules of the Leguminoseae.

It has already been pointed out that the presence or absence of nitrite in the expressed sap of plants may be difficult to prove in view of the fact that the sap is a mixture of substances, some of which (e.g. anthocyans) give a pink colour reaction with Griess-Ilosvay (which therefore does not necessarily indicate nitrite), others of which (e.g. tannins) may combine with the nitrite on the contact inevitable on expression. It was, moreover, found in more than one instance that an extract which gave a slight positive reaction when quite fresh, failed to do so on standing for a few hours (e.g. *Sambucus*). In other examples, e.g. *Allium* and *Rhamnus*, it was found that mechanisms were present which could not only prevent the detection of nitrite in the sap but could further render impossible, by means of the Griess-Ilosvay reagent, the detection of nitrite added to the extract. This problem is still under investigation.

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[1] Molisch, op. cit., 89.

[2] Czapek, op. cit.

[3] Aso: Beihefte Bot. Cent., xv. 208, and xxxii. 146.

[4] Klein, op. cit.

The freshly expressed sap of the shoots of 105 species has been examined, in only 25 of which has the Griess-Ilosvay reaction given positive results. These are as follows:

Good reaction.	Weak reaction.
Bryonia dioica	*Arum maculatum*
Centaurea nigra	*Helianthus annuus*
Helianthus tuberosum	*Lupinus*, sp.
Lythrum Salicaria	*Lychnis alba*
Physalis Alkekengii	*Medicago lupulina*
„ *peruviana*	*Pastinaca sativa* (very weak)
Reseda lutea	*Populus nigra* (very weak)
Solanum crispum	*Rubus Idaeus*
„ *Dulcamara*	*Sambucus nigra*
„ *tuberosum*	*Silene maritima*
Ulmus campestris	*Solanum Lycopersicum*
	„ *nigrum*
	Urtica dioica
	Veronica Chamaedrys

The presence of nitrite was also shown in etiolated pea seedlings.

That the nitrite content may vary considerably in a species was shown in several instances, and conspicuously in *Silene maritima* and *Sambucus nigra*, but as yet the evidence is insufficient to show whether the determining factors are similar to those which probably obtain in the case of nitrate.

Conclusion. Notwithstanding that the present paper is in the nature of a preliminary communication, a few remarks of a general nature may not be out of place, more especially relative to the presence of atite in certain plants. This substance might be regarded as the mechanism for the reduction of the initial nitrate in protein synthesis; certainly the temptation to do so exists, but it must be remembered that atite, as far as observation goes, is of rare occurrence, and its activity at ordinary air temperatures, even in the presence of a potent activator, is low; so low, in fact, that the result can only just be detected after a lapse of about five hours. It is only in the artificial conditions of high temperatures (45° C.) and in the presence of acetaldehyde that its activity gives measurable results. For this reason the evidence available does not point to the conclusion that it plays any important rôle in the elaboration of protein.

SUMMARY.

1. The presence of nitrate is shown to be a not uncommon feature in the green plant. The amount present is shown to vary according to different conditions of growth, and the occurrence of nitrate in a given species is shown to be inconstant.

2. A nitrate-reducing mechanism, termed atite, has been shown to occur in 23 plants.

3. The atite of the potato is a thermolabile and oxidizable substance, which, under the conditions of the experiment, can only convert a limited amount of nitrate to nitrite.

4. The presence of acetaldehyde may affect the activity of the atite of different plants in different ways. More than a single mechanism is possibly involved.

5. The presence of nitrite was found in more than 20 per cent. of the plants examined.

6. The evidence does not point to the conclusion that atite is of much significance in the protein metabolism of the plant.

In conclusion, I wish to express my thanks to Mr. T. G. Hill, under whose supervision this work was done.

The Distribution of Certain Portions of the British Flora.

II. Plants restricted to Scotland, England, and Wales.

BY

J. R. MATTHEWS, M.A., F.L.S., F.R.S.E.,

Royal Botanic Garden, Edinburgh.

With five Diagrams in the Text.

INTRODUCTION.

A CLOSE relationship invariably exists between the flora of a continental island and that of the nearest mainland, a good example being provided by the British flora itself. For a general discussion of such island floras, their origin and distribution, one need only turn to Darwin's 'Origin of Species' or Wallace's 'Island Life'. In these classics the theory of migration is advanced as a sufficient and satisfactory explanation of the known facts of distribution. Darwin's views are strikingly summarized in the following sentence: 'If the difficulties be not insuperable in admitting that in the long course of time all the individuals of the same species, and likewise the several species belonging to the same genus, have proceeded from some one source, then all the grand leading facts of geographical distribution are explicable on the theory of migration, together with subsequent modification and the multiplication of new forms.' There is no need here to quote more than one illustration of this great generalization. The distribution of *Salix* in South Africa has been described recently by Burtt Davy (1922), who regards the South African species of the genus as modern types descended from an ancestral form, *Salix safsaf*, Forsk., of north and tropical Africa which has migrated southwards along fairly definite paths, producing new forms on the way, nearly all of which are endemic.

But the evidence for a single centre of development and dispersal is not

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always so clear. So great is the discontinuity of area of certain species that several writers have advocated the theory of polygenesis for these puzzling cases in preference to the Darwinian point of view. Bews (1921), writing of the flora of South Africa, admits the possibility of polygenesis, 'for if it affords a simpler explanation than ordinary migration over enormous areas, then the onus of proof is on the side of those who deny the possibility of multiple origins.' From quite another quarter, Holm (1922), dealing with the geograpical distribution of arctic plants, also favours the view that one and the same species may arise in more than one single area and that a genus may develop from several centres. The question has been fully discussed by Clements (1904), and, in fact, more than a hundred years ago Schouw (1816) supposed that very often the same species must have been produced in different, widely separated localities. It is interesting that recent studies, supplied with a greatly augmented number of facts relating to geographical distribution, should result in a revival of this old view, yet, whatever the future verdict regarding certain outstanding difficulties, the theory of migration must always stand as accounting for all the 'leading facts' of plant-geography. It can never be seriously challenged, least of all when a comparatively small part of the earth's surface such as Britain, with its known geological history, is the area under consideration. For the flora of Britain is almost wholly an outpost of the larger European flora, and, fragmentary though the fossil record be, there is reason to believe that our native flora has attained its present range in the country as a result of past migrations brought about largely by climatic change. With the historical factor becoming more clearly defined, these distribution studies were commenced in the hope that some light might be shed on the several plant invasions from the Continent which have shared in the building up of the flora of Britain, and to ascertain, also, how far the 'Age and Area' theory of distribution propounded by Dr. Willis might be applicable to our rather complex flora.

In my former paper (1923) reference is made to the researches of Clement Reid, continued by Mrs. E. M. Reid, on Pliocene floras. The comparative review given by Mrs. Reid (1920) seems to prove conclusively that the flora of Western Europe underwent a gradual change during the Pliocene until there emerged, towards the close of the Pliocene, a vegetation composed almost entirely of indigenous species of the present time. This conclusion is substantiated by the investigation of Depape (1922) on the Pliocene flora of the Rhone valley. In the south-east of France a striking change occurs between the Plaisancien flora, rich in exotic species, and the quaternary flora, composed very largely of indigenous species, a contrast which is explained by the geological and climatic changes which marked the end of tertiary and the beginning of quaternary time. The question which has given rise to so much difference of opinion is whether the oncoming cold of the

Pliocene, culminating in the Pleistocene, exterminated the existing flora within the limits of Britain, or whether a considerable portion survived the glaciation of the country.

A consideration of the known composition of floras from pleistocene and recent deposits described by Reid (1899, 1913, 1916), Lewis (1905–1911), and Chandler (1921) suggests that few of our southern species survived the period of maximum cold even in the south of England, which was ice-free. Since my former paper was written, Chevalier (1923) has published a com-

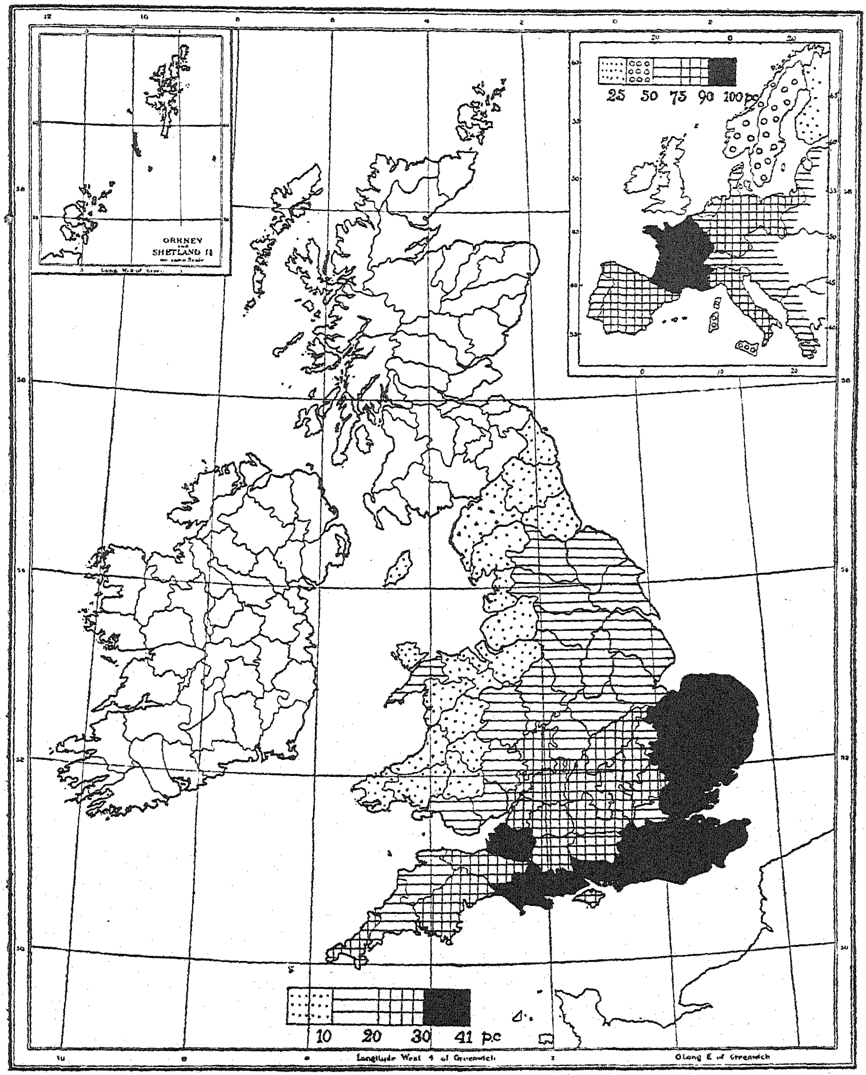

DIAGRAM I. Distribution of 266 plants of the British Flora confined to England and Wales.

parative study of the vegetation of North-west France and Great Britain, and points out that the present differences cannot be explained on climatic grounds, but believes that the northward migration of species after the retreat of the ice had not terminated when the Channel was opened. Thus a barrier was formed which prevented the penetration of numerous continental species into England.

In the case of species having a southern or south-eastern distribution in England, the possibility of tracing connexions with continental sources of dispersal is illustrated in my paper already mentioned dealing with that portion of the British flora restricted to England and Wales. Without seeking to establish the relative importance of such invasions as are there suggested a distinction is drawn between a migration from the east and another from the south, and it is believed that along these lines and between them the bulk of the English flora has probably advanced. In Diagram 1, the general map illustrating the mass distribution of these 'English' plants is repeated, since it may aid in explaining a further analysis of our flora presented in the following pages.

This paper deals with a small portion of the British flora which is found in Scotland as well as in England and Wales but not in Ireland, at least as indigenous plants of that country. For convenience, the 105 species which form this group may be styled Anglo-Scottish, and a cartographic presentation of the distributional data relating to them may be useful in supplementing what has already been attempted for entirely 'English' species.

DISTRIBUTION OF 105 ANGLO-SCOTTISH SPECIES.

It should be stated at once that the status of some of these species in Scotland is in doubt. They are probably adventitious and not really native in some of their northern stations, their occurrence being due to human agency. For the purpose of indicating the range of the group as a whole, however, I have used the topographical statistics furnished by Watson (1883) and Bennett (1905), and in certain cases I have had the advantage of the most recent information in Mr. Bennett's possession.[1]

The topographical data relating to these 105 species taken together are expressed cartographically in Diagram 2. The group is widely dispersed in Europe, yet the maximum number of species appears in France and

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[1] Many questions in plant distribution would be less puzzling if a fuller record of man's influence on the flora were available. No serious student of British plants doubts that many species included in descriptive Floras as if they were native in the country are in all probability not so. It is the difficulty to disentangle the truly indigenous, i.e. aboriginal or autochthonous, from the adventive that necessitates and justifies the careful recording of those immigrants that are establishing themselves at the present time. In this connexion such works as Dunn's 'Alien Flora of Britain' and Trail's 'Flora of the City Parish of Aberdeen' may be consulted, while, for another part of the world, Thomson's 'Naturalization of Animals and Plants in New Zealand' is most interesting.

Germany. Consequently, if Central Europe has been the chief centre of dispersal, one might expect in Britain a concentration in the south and south-east with a gradual diminution in numbers northwards, an extension, in fact, of the main features which characterize the range of entirely

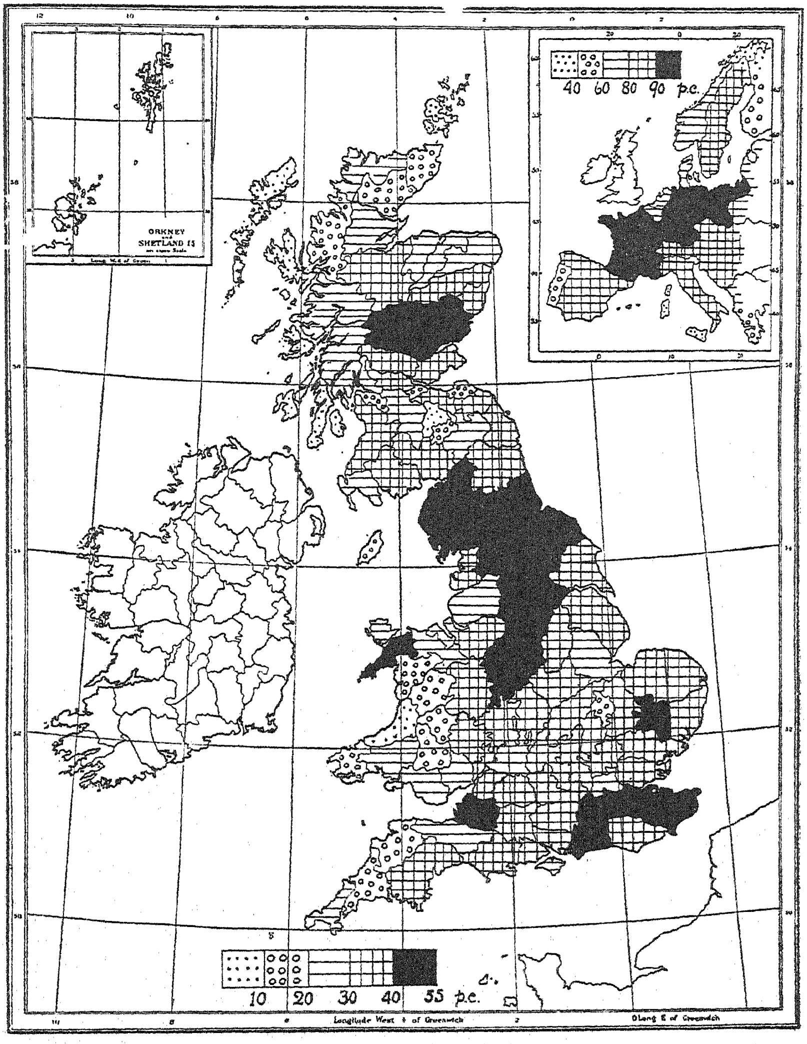

DIAGRAM 2. Distribution of 105 'Anglo-Scottish' species of the British Flora.

'English' plants shown in Diagram 1. But the general result showing mass distribution is here different. The distribution within Britain is uneven, exhibiting several areas of density in England, while in Scotland the maximum numbers appear in Perth and Forfar.

To discuss fully a general map such as Diagram 2 would necessitate a critical consideration of the individual species, and in our flora there is ample scope for these detailed studies in distribution. Recently Miller Christy has dealt with *Primula elatior*, Jacquin (1922), and *Carpinus Betulus*, Linn. (1924). But for the broad outlines which are here intended it suffices to recognize two main types of distribution, a northern and a southern. Of the 105 plants admitted, 50 are found most frequently in Scotland and in the north of England, while 55 tend to be more southern. The latter occur, on the average, in 38 of the 71 Watsonian vice-counties in England, and in 7·4 of the 41 in Scotland, while for the former the figures are 6·5 and 12 respectively. The numbers alone give a good idea of the relative extent of areas occupied by the two groups of plants in the two countries.

Some of the boreal species may be described as arctic, whether we follow the view of Hooker (1862), who accepts all species as arctic if they are found growing within the Arctic Circle, or the more recent one of Ostenfeld (1902), who makes the tree limit the line of demarcation. Others which do not reach high latitudes are, nevertheless, characteristic of northern or montane floras, and in Britain certainly exhibit a northern tendency. This boreal element in the Anglo-Scottish group of plants will be referred to later; meanwhile the remaining 55 non-boreal or more southern species may be dealt with.

DISTRIBUTION OF FIFTY-FIVE SOUTHERN SPECIES OF THE ANGLO-SCOTTISH GROUP.

These are mapped by themselves in Diagram 3, and while many of them extend into southern Scandinavia, most are representative of the central and south European flora. Five are classed as 'southern' members of the British flora by Stapf (1914), i. e. they do not enter into the composition of the flora of Central Europe, but have a distribution which is essentially south-western. These are *Brassica monensis*, Huds., *Ulex nanus*, Forst., *Daucus gummifer*, Lam., *Genista anglica*, Linn., and *Limonium vulgare*, Mill. To these may be added *Polygala calcarea*, Schultz. They may be regarded as having become constituents of our flora by migration from the south, thus linking up with 'Peninsula' and 'Channel' species of the entirely 'English' group, but they differ from these in ranging over a wider area, since they have penetrated northwards into Scotland.

It will be seen from Diagram 3, however, that the majority of these 55 species are found most generally in the south-eastern part of England, from the Bristol Channel to the Humber. In Europe over 90 per cent. of the number is recorded from France, Spain, and Belgium, *Linum perenne*, Linn., and *Potamogeton falcatus*, Fryer, being the only two members of the group not reported from France. If re-immigration into Britain from this part of the Continent is postulated, a heaping up of species in south and south-east England is not unexpected. It would almost appear, in fact, that Diagram 3 could be interpreted in terms of Diagram 1. Allowing for minor fluctuations, the same general features are obvious, notably the concentration of species in the south-east with a thinning out westwards and northwards. But in the case of the southern members of the Anglo-Scottish group a greater extension of range has been effected, so that they appear over a larger part of England and extend into Scotland as well.

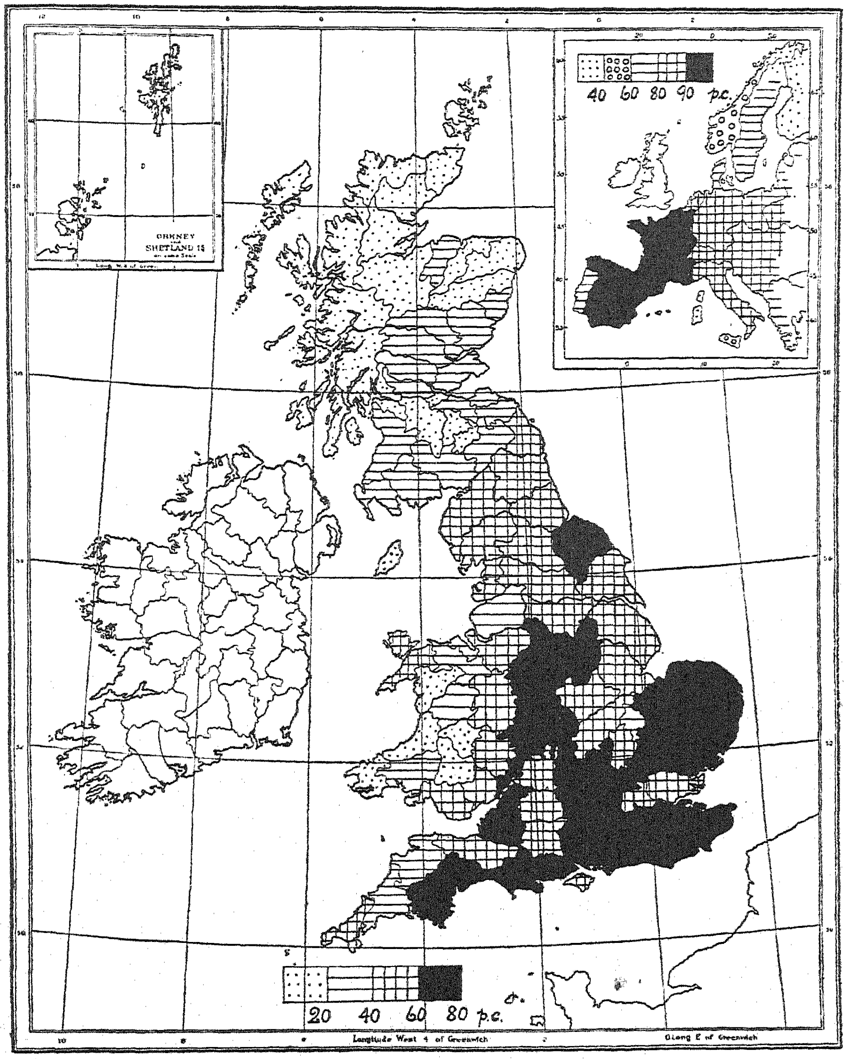

DIAGRAM 3. Vice-comital distribution of fifty-five southern species of Anglo-Scottish element.

While the general trend of migration is obvious, the precise directions from which these species may have reached Britain, and the points at which colonization was first effected, are more difficult to determine. Only when plants are at a relatively early stage in the process of spreading over a given area is it possible to trace with any degree of certainty their lines of dispersal. There are a number of relatively thinly populated districts in the otherwise rich south-eastern half of England, as mapped in Diagram 3, which may indicate larger steps than are general in the process of spread or local extermination within areas once occupied—an event which must not be overlooked despite the small number of recent plant extinctions in Britain, about six according to Druce (1920), since 1597.

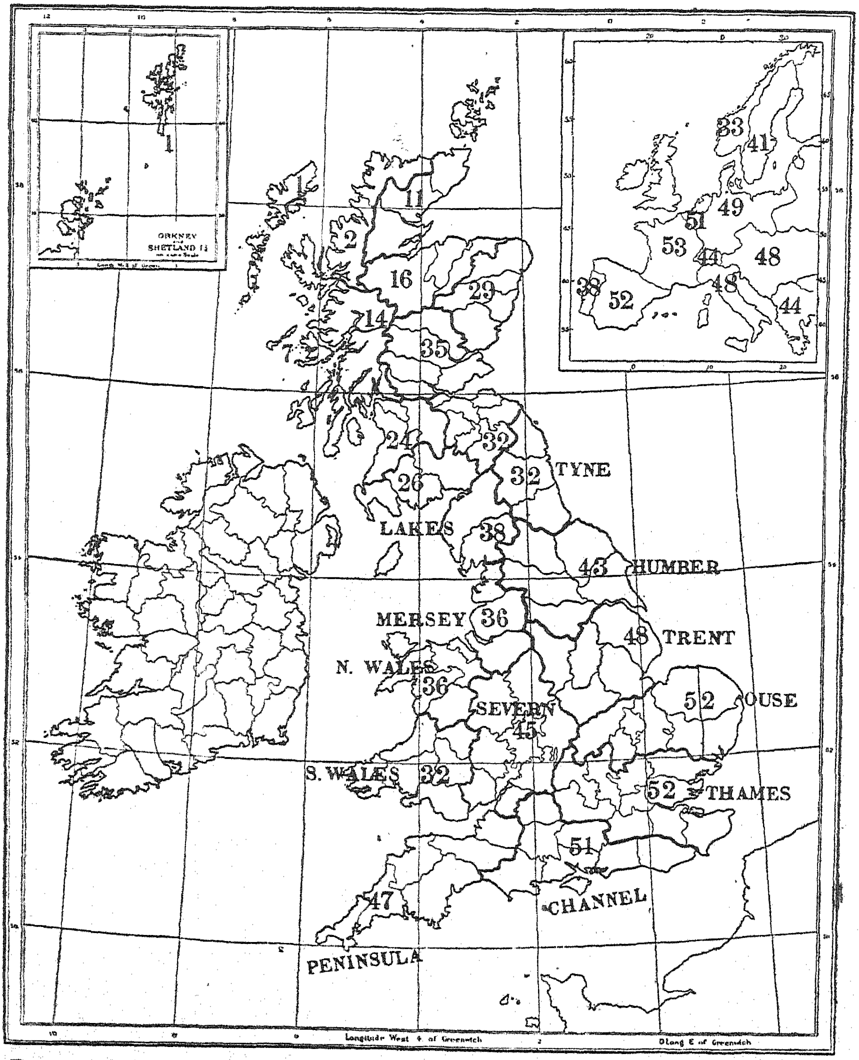

DIAGRAM 4. Provincial distribution of fifty-five southern species of Anglo-Scottish element.

If, however, larger areas than Watsonian vice-counties are employed, the geographical connexions become clearer. Diagram 4 has been constructed for this purpose. All but one of the 55 southern species occupy the Thames and Ouse provinces taken together, the absent member being *Brassica monensis*, Huds., a western species extending from South Wales to Arran and Cantyre. Of the remaining 54, *Centaurium vulgare*, Rafn., and *Potamogeton falcatus*, Fryer, have not been recorded from the Thames province, and *Cardamine bulbifera*, Crantz., and *Daucus gummifer*, Lam., are absent from the Ouse, although the latter (essentially a western species) occurs as an outlier on the Yorkshire coast. The Channel province is almost as rich with 51 species, while the Trent area comes next with 48. The Peninsula province, with 47 species, has an almost equally large number, while the Severn has 45 and the Humber 43. All the other provinces or sub-provinces, as may be seen from Diagram 4, have each fewer than 40 of the 55 species admitted, the numbers falling markedly in the north and west of Scotland. In general terms the figures in Diagram 4 agree with Dr. Willis's hypothesis of 'Age and Area', providing a further instance of the applicability of that theory of distribution to a particular portion of the flora of Britain.

Regarding the distribution of these 55 southern species during Pliocene and Pleistocene times, we have, unfortunately, very little information. Only one member of the series, *Stellaria aquatica*, Scop., is known to have existed in Britain during pre-glacial and glacial times. This plant is widely distributed in Europe, and in Britain it occurs as far north as Stirling, but is absent from the south and west counties of Wales. Its association with arctic species in late glacial deposits may point to survival during the rigours of the Ice Age, at least in South Britain, yet there can be little doubt that it, along with others, has advanced northward since the ice-sheet receded. But whether the source of dispersal during the post-glacial period was within England or beyond it, the majority of the 55 species we are considering have now come to occupy the whole of the south-eastern part of the country, for 41 of them are common to the five provinces—Peninsula, Channel, Thames, Ouse, and Trent. It is this width of range, as has already been pointed out, that makes it impossible to determine the exact migratory paths which these species have followed. But if dispersal be a gradual process, working even roughly according to the 'Age and Area' principle, there should be some slight indication of the scheme in the distribution of the remaining 13 species[1] throughout this area. Their range is as follows:

7 occur in four provinces;
5 occur in three provinces;
1 occurs in two provinces.

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[1] *Brassica monensis* is absent from the area.

Although the number is small, it may be worth while to examine the distribution of these 13 species a little more closely. None of them is common to the Peninsula and Trent, the most widely separated districts of the area under consideration. The Ouse and Thames provinces have each 11 of the 13 species, the Channel has 10, the Trent 7, and the Peninsula 6. The figures showing the relationships among the five provinces specified are set out in Table I.

TABLE I.

Trent.	*Ouse.*	*Thames.*	*Channel.*	*Peninsula.*
7	7	6	4	0
7	**11**	9	8	4
6	9	**11**	9	5
4	8	9	**10**	6
0	4	5	6	**6**

The 'Age and Area' hypothesis could be applied to these figures since each set shows a decrease from a maximum, but the numbers are too small in themselves to provide a basis for argument. They are of interest, however, in so far as they fall into the general scheme illustrated for four provinces in my earlier paper dealing with the much larger assemblage of plants restricted to England, and here given for comparison.

Ouse.	*Thames.*	*Channel.*	*Peninsula.*
142	108	101	63
108	**144**	116	73
101	116	**157**	89
63	73	89	**117**

Such findings suggest certain areas of establishment and subsequent spread in different directions. Thus, from Table I, the 7 Trent species might be expected to have had an eastern centre of dispersal, while the 6 Peninsula species are more likely to have arrived from the south. This is borne out in a general way, though not with absolute certainty in every case, from an examination of the continental and British distribution of the several species, the 6 Peninsula plants being *Cardamine bulbifera*, Crantz., *Silene conica*, Linn., *Ulex nanus*, Forst., *Daucus gummifer*, Lam., *Centaurium vulgare*, Rafn., and *Glyceria procumbens*, Dum., while the 7 Trent species are *Fumaria Vaillantii*, Lois., *Arabis perfoliata*, Lam., *Linum perenne*, Linn., *Arnoseris pusilla*, Gaertn., *Myosotis sylvatica*, Hoffm., *Potamogeton zosterifolius*, Schum., and *P. falcatus*, Fryer.

With these indications of dispersal in different directions within England it would not be surprising to find (since we are dealing with plants that have extended into Scotland) that some have moved northwards along the east coast, while others may have extended their range in a north-west direction. Diagram 4 again indicates the main outlines, and if we examine the data from this point of view, we find that, of our 55 species, 15 are reported from the eastern counties of Scotland only, 9 from the western only, and 31 are common to both. The general trend of the northward migrants is thus indicated, some reaching Scotland in the south-east lowlands, others finding their way into the south-west of the country. A gradual extension northwards can be detected for the majority, for they show little or no discontinuity of area. On the other hand, a few do exhibit discontinuous distribution. In some instances this may be due to an adventitious origin, since such plants as *Fumaria Vaillantii*, Lois. (recorded from Linlithgow), *Chenopodium vulvaria*, Linn. (northwards to Fife), and *C. polyspermum*, Linn. (Berwickshire), are found on cultivated ground or in waste places. But the circumstances seem different in the case of other species. *Hypericum montanum*, Linn., illustrates a relatively small gap between English and Scottish stations, the plant being fairly general in England northwards to Durham and Cumberland, appearing in Scotland in Ayrshire. *Cardamine bulbifera*, Crantz, a local woodland species in South-east England, has an outlying station in Stafford and is regarded as native in Ayrshire. The range of *Ulex nanus*, Forst., can be traced from the south of England to Cheshire, North Wales, Isle of Man, Kirkcudbright, and Dumfries, but its occurrence in Caithness (recently reported as an apparently indigenous plant there) affords an instance of a rather large gap in an otherwise fairly continuous south to north range. In Europe, the species is native in France, Spain, and Portugal. Again, *Polygala calcarea*, Schultz, is generally distributed in the south of England, whence it takes a big step to West Sutherland. It is a plant of dry chalky places, and on the Continent it is essentially south-western in its distribution.

These few examples, illustrating varying degrees of discontinuity, serve to check any assumption that all species spread outwards and establish themselves from some centre of dispersal by small and gradual stages. They point rather to the establishment of the same species at isolated localities within a given region, thus rendering possible further spread from more than one area. In this way, doubtless, some of what are now our commonest plants have become dispersed throughout the whole of Britain, and the process may be followed at the present time by tracing the progress of recently arrived aliens.

These observations naturally lead to the 'single species' criticism of Willis's theory of distribution, for the application of which its author holds that groups of ten, at least, must be dealt with, never a single species. The theory, especially some of its later developments, has been freely discussed and criticized, but the simple idea with which the hypothesis commenced has not been disputed. In broad outline the distribution of numerous floras has been shown to agree with the general principle that the longer a group of species exists the greater will be the area occupied. Moreover, if the flora be limited, such as the 'English' element in the British flora or the non-boreal element among Anglo-Scottish species, the analyses appear more convincing. But the entire British flora as it exists to-day does not conform to the general rule, and, as might be expected, among the most prominent exceptions are the alpine and arctic species which constitute the northern and montane element of our flora, although Willis (1923) suggests that at its time of spreading this element also followed the 'Age and Area' rule.

DISTRIBUTION OF FIFTY BOREAL SPECIES OF THE ANGLO-SCOTTISH GROUP.

The mass distribution of those species recorded from Britain but absent in Ireland, which are here classed as boreal, is shown in Diagram 5. Naturally it is to the highlands of Scotland that one looks for the greatest concentration of these plants, although considerable numbers are found in North England and North Wales. All occur in Scandinavia except *Crepis succisaefolia*, Tausch., and *Symphytum tuberosum*, Linn., both of which certainly exhibit a northern tendency in Britain, even if they cannot be termed arctic-alpine. As many as 38 are reported by Norman (1895) from the north of Norway within the Arctic Circle. Not a few are circumpolar, being known from North America, Greenland, Arctic Europe, and North Asia. Generally speaking, they represent a portion of what has been called the Scandinavian element of our flora, the existence of which in Britain was attributed by Forbes to migration from Scandinavia during the glacial period. But most of these plants reappear in Central Europe—only one, *Cerastium arcticum*, Lange, being absent—leaving a considerable intervening area where many of them are unknown. The increasing fossil record (Chandler 1921) goes far to show that they did exist within this area during the Pleistocene, and this recent evidence adds substantially to that derived from present distribution in support of the old, well-known theory of a former widespread southward migration. It becomes a question, however, whether the occurrence of arctic plants in Britain is due to their becoming stranded *during* the migration from the north, or to re-introduction from non-glaciated parts of Europe *after* the retreat of the northern ice-sheet. It is a further question whether some of our boreal plants did not have their origin in the alpine districts of Central Europe, wherefrom they accompanied originally arctic species in the homeward migration when the ice receded. Much information is necessary before these questions can be discussed fully.

Not infrequently the areas in which a number of our boreal species occur are of limited extent, although a line drawn round the whole region within which the plants are known to grow would in some instances include the greater part of the Northern Hemisphere. This is taken to indicate age, and the boreal members of the Anglo-Scottish group of plants are regarded, on the average, as older within Britain than the southern members of the group. Disintegration of area there has been, the result of climatic change, these fluctuations being well described in a recent book by Brooks (1922). The post-glacial changes in the vegetation have been fully studied by Lewis (1905–11), whose investigations show that since the close of the Ice Age climatic conditions have varied sufficiently to favour at one time an

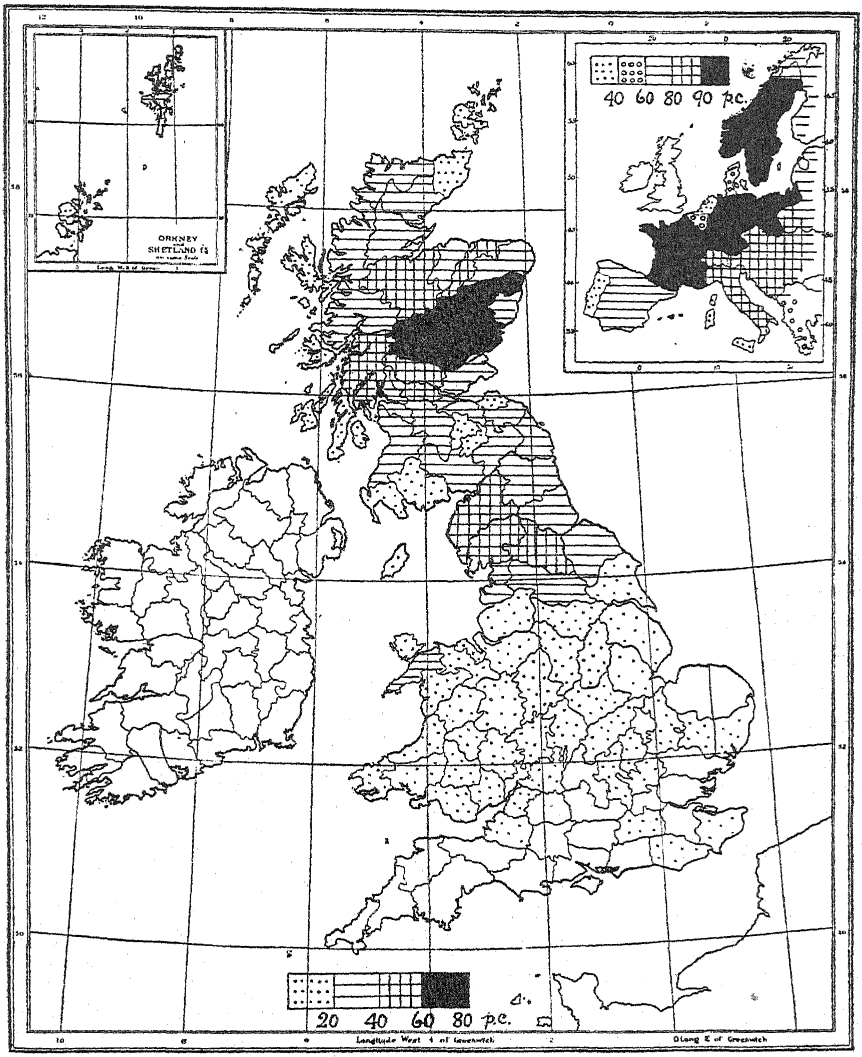

DIAGRAM 5. Distribution of boreal species of Anglo-Scottish element.

extensive development of forest, at another a widespread formation of peat. It is known that changes in the distribution of the Scots Pine, for example, have followed these fluctuations in climate, and doubtless sylvestral herbs were also affected. One knows that within recent times the disappearance of forest has led to the extinction in certain localities of *Linnaea borealis*, Linn., *Trientalis europaea*, Linn., *Corallorrhiza innata*, Br., and *Goodyera repens*, Br., to mention just a few characteristic British boreal species. Again, changes in peat bogs, effected naturally or artificially, have unquestionably modified the distribution of helophilous species, e.g. *Scheuchzeria palustris*, Linn. Thus, while the glacial epoch was the means of adding a considerable arctic element to our flora, the post-glacial period has witnessed much disintegration of that element, certain members becoming exterminated; while others have been brought to the verge of extinction within the boundaries of Britain.

SUMMARY.

This paper is the second of a series dealing with the distribution of certain limited portions of the British flora. It treats of 105 species which are found in Great Britain but absent from Ireland.

A few remarks are made on the general question of migration, since the theory of migration is accepted as accounting for the close resemblance of our flora, as a whole, to the flora of Europe.

In the case of the 105 species with which the paper deals, the general trend of migration from the Continent is indicated most clearly for those members of the group, 55 in number, which exhibit a southern tendency within Britain. Their distribution is essentially similar to that of the larger assemblage of entirely 'English' species previously dealt with, except that they are more widely distributed and it becomes difficult therefore to determine lines of invasion. Certain details are given for a small number somewhat restricted in their range, and these suggest, as before, points of invasion and establishment round our south and east coasts.

The extension northwards into Scotland is then briefly considered. Most of the species show a fairly continuous range, but some exhibit considerable gaps between their English and Scottish stations. This may result in spread from more than one centre, and in different directions, leading to complications in terms of the 'Age and Area' principle when a flora is taken as a whole.

The remaining 50 species, found most frequently in Scotland, are classed as boreal. In respect of their occupation of the country, they are regarded as older than the 55 southern species. The majority of them are arctic, and a number are known from glacial deposits in South Britain, where they may have survived the rigours of maximum glaciation or, if they migrated

farther south, they were re-introduced when an arctic-alpine flora moved northwards on the retreat of the ice-sheet. Post-glacial fluctuations of climate have led to localization of many of our boreal plants, so that they now show discontinuity of area.

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The Formation of Plant Growth-promoting Substances by Micro-organisms.

BY

FLORENCE A. MOCKERIDGE, D.Sc., F.L.S.

From the Department of Biology, University College of Swansea.

THE attention which has been attracted during the past few years to the subject of vitamines in connexion with animal and human nutrition has resulted in the conclusion that, since the animal is ultimately entirely dependent on the plant for its food, it is similarly dependent for its vitamines, which are apparently synthesized in the main by the green parts of plants (10), although they also appear to be present in chlorophyll-free parts such as onion root, and in chlorophyll-free plants such as *Agaricus campestris* and *Monotropa uniflora* (25). This work has had the effect of directing a certain amount of attention to plant nutrition from the point of view of accessory food factors, and although the methods of nutrition of the plant and the animal differ so fundamentally in character, there arose the suggestion, based on numerous observations, that growth-promoting substances may play an important part in plant nutrition.

The researches of Bottomley (3, 4, 5, 6, 7, 8, 9) have established the fact that small quantities of organic materials have a very beneficial effect on the growth of various plants. In fact, the results of his work go farther and indicate quite clearly that, without the organic materials, the plants with which he worked cannot attain their maximum development.

Acknowledgement of the value of organic materials in plant nutrition appears to be the result of the observations of other workers. Allen (1) working with the plankton diatom *Thallassiosira gravida*, Cleve, has shown that a satisfactory culture cannot be obtained in an artificial 'sea-water' obtained by dissolving, in the requisite quantity, the inorganic salts which are found in the natural sea-water. Something essential to healthy growth is lacking from such a medium, and, in the words of the author, it is quite probable that the deficiency is a 'somewhat stable organic compound', which is apparently supplied by a small addition of natural sea-water. Linossier (17) pointed out that *Mycoderma vini* showed a marked acceleration of growth on the addition of 'vitamines' to the culture medium, and that other fungi, as *Oidium lactis*, respond to the addition of such substances in orange juice, especially when grown in a deficiency of nutrients. The requirements of *Saccharomyces* in this connexion have been the subject

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of much discussion since Williams (30) announced that this organism is a source of the animal vitamines. The publications of Bachman (2), Fulmer, Nelson, and White (14), Nelson, Fulmer, and Cessna (24), Fulmer, Nelson, and Sherwood (13), MacDonald (19), MacDonald and McCollum (20), Heller (16), Funk and Dubin (15), and Eijkman, van Hoogenhuijze, and Derks (11), all deal with the question whether the organism synthesizes the whole of the vitamines which it contains, or whether it requires some initial supply in the culture medium; and this point must be regarded for the time being as unsettled.

In connexion with the higher plants, Robbins (26), experimenting with corn root-tips, found that for their proper growth some substance is required which is not found in an ordinary nutrient solution, but which can be supplied by the addition of peptone or autolysed yeast, but not by a carbohydrate. Livingston (18), in a series of experiments on the effect of manure extracts on plants growing in water culture, admitted that the organic material was a most important factor, and Wilkins (29) has much more recently stated that there is at present in organic manures some important organic factor over and above the ordinary inorganic chemicals from which marked benefit to the crop is derived. The author (21 and 22) has shown that these important organic materials not only affect the soil bacteria, but are also most probably the products of bacterial action in the organic material of manures, for organic substances which had the power of promoting growth were extracted from all the well-known and frequently used organic fertilizing materials, and the greater the degree of bacterial decomposition which the material had undergone the greater was the proportion, as measured by their effect, of these substances, which Bottomley (5) has called 'auximones'. In all probability, therefore, the normal green plant obtains its growth-promoting substances from the soil organic matter, from which they are liberated by bacterial action; by their aid builds up its own body substance, in the course of which process it manufactures the 'vitamines', which are then passed on to the animal in the form of plant food. The work of elaborating the growth-promoting substances is therefore thus relegated to the soil bacteria.

The question arose as to whether these plant-auximones are actually products of the bacteria themselves, i.e. manufactured in their own bodies and excreted to the exterior, or whether they are merely degradation products of the organic débris in which the bacteria are working. In this connexion Bottomley (7) has shown that a crude extract of ordinary peat, which contained derivatives of nucleic acid, as well as small quantities of the sodium salt of the acid itself, presumably derived from the nuclei of the plants originally composing the peat, has an auximonic effect on the growth of plants in water culture. The author (22) has similarly found that all the extracts of organic manures which had a growth-promoting effect contained also these nucleic acid derivatives, and the greater the effect shown, the greater was the proportion of these materials. Obviously it does not follow that these substances are the auximones, nor, if they have a growth-promoting effect, that they are the only ones, for all the liquids so tested were crude extracts of complex organic composts, which would contain a variety of materials; but, on the other hand, it seems not at all unlikely that these substances would have the effect of increasing the growth of the plant, since the most marked result on the appearance of the tissues of the plants supplied with these materials is shown in the increased size of the nucleus, of which nucleic acid is an important constituent. It therefore appears that the organic matter of the soil contains degradation products which have a growth-promoting effect on ordinary green plants, this effect being especially marked on the nucleus.

On the other hand, some, at least, of these auximone materials may be directly products of the bacterial metabolism, which are excreted by the organisms and absorbed by the plants. That this is so was proved by adding the sterilized cultures of certain bacteria to the culture solutions in which plants were growing (7). The nitrogen-fixing bacteria, *Azotobacter chroococcum* and *Bacillus radicicola,* were tested in this way, and it was found that certain products of these bacteria have a growth-promoting effect on green plants.

The first striking results which led, in the case of Bottomley and the author, to the original conception of the probable functioning of accessory food substances in plant nutrition, were obtained with a material prepared by treating peat with certain decomposition bacteria, followed by nitrogen-fixing organisms. Small quantities of the watery extract of this material, when added to the usual solutions of inorganic salts used for the growth of plants in water culture, gave increases in growth equal to many hundred times that which could be accounted for by the effect of the additional actual food materials present. There were present in this material, however, substances which might act as auximones which were derived from two sources—the peat and the bacteria. It appeared possible that ingredients from either or both of these sources might function as auximones; and, further, it seemed reasonable to suppose that if the nucleic acid derivatives have a stimulating effect on plant growth, they most probably function mainly as nutrients in that they supply small quantities of materials essential to nuclear elaboration, rather than as auximones in the accepted sense, functioning in a similar way to the vitamines.

It was assumed that in the case of a material acting as a nutrient, the effect produced on the rate of plant growth would be proportional to the amount of this ingredient supplied; while, on the other hand, in the case of a material functioning in the same way as the vitamines in animal nutrition, so long as the necessary minimal quantities of the auximone be present, any further additions would probably not result in a proportional increase in effect, though such additions would probably operate to a certain extent.

In order to test in this way the probable function of both the crude nucleic acid derivatives and the products of the nitrogen-fixing organisms, a series of culture experiments was made with *Lemna minor* on the lines which have been set out previously by Bottomley and the author.

This particular plant was chosen because, in the first place, it is a water plant, and therefore, in growing in water culture, it would be growing under approximately normal conditions; and, in the second place, it propagates itself vegetatively so rapidly that a fairly accurate measure of the rate of growth can be obtained by counting the shoots.

To carry out the experiment, seven series consisting of five round, flat, glass crystallizing dishes were arranged, into each of which was put 150 c.c. of Detmer's culture solution, containing all the essential inorganic ingredients for plant growth. Series I, containing dishes Nos. 1–5, served as a control set, for here the plants were growing in inorganic materials only. To each of the dishes of Series II, which were numbered 6–10, were added the crude nucleic acid derivatives from raw peat in the proportion of the extract from 1 grm. of peat per 250 c.c. of solution, or 4 grm. per litre. To the dishes of Series III and IV, numbered 11–15 and 16–20, was added a similar material in the proportion of the extract from 1 grm. of peat per 500 c.c., or 2 grm. per litre, and the extract of 1 grm. per litre respectively. To Series V, VI, and VII, numbered respectively 21–5, 26–30, and 31–5 was added a culture of *Azotobacter* in similarly varying proportions. In order to obtain this, a pure culture of *Azotobacter chroococcum* was grown upon an agar medium and the gelatinous growth carefully scraped off and weighed. It was then sterilized by heating in the autoclave at a temperature of 140° C. for three successive periods of half an hour each, with two-hour intervals. Sterile distilled water was added, and the whole mass thoroughly broken up by vigorous shaking in a shaking machine for several hours, until a uniform suspension was obtained. Chloroform was added to the material to prevent bacterial contamination. Aliquot portions of this suspension were then added to the various dishes in the separate series, the chloroform having been evaporated by gentle heating, so that Series V contained 1 grm. of the gelatinous *Azotobacter* growth in every 500 c.c. of culture solution, or 2 grm. per litre; Series VI contained 1 grm. per 1,000 c.c., and Series VII, one grm. per 2,000 c.c., or 0·5 grm. per litre. Ten similar plants of *Lemna minor* were then counted out into each dish, and the dishes were covered, to the level of the liquids, with paper which was black on the inner side and white on the outer, in order to cut off light from the bottom and sides of the dishes, but at the same time to avoid the rise in temperature which would follow the absorption of heat by uniformly black paper.

At the same time, three hundred similar plants were counted out, thoroughly washed, placed in a tared vessel and dried at 100° C. until their weight was constant. In this way a measure of the average weight per plant at the beginning of the experiment was obtained.

The whole series of dishes was placed in a greenhouse and kept going for nine weeks. In order to prevent, as much as possible, bacterial action and the growth of small algae, as well as to maintain uniformity in the concentration of the solutions, the latter were changed twice weekly, the plants being carefully washed at the same time with conductivity water. All solutions were made up and all operations carried out with this conductivity water, in order to avoid any possible toxic effects from ordinary distilled water. Once weekly, at the time of changing the solutions, the plants were counted, and at the end of the third week they had increased in number to such an extent in some of the series that they were quartered throughout the set, only one-fourth of the produce from the original ten in each dish

TABLE I.

		No. of Plants at End of								
Series.	*Dish No.*	*1st wk.*	*2nd wk.*	*3rd wk.*	*4th wk.*	*5th wk.*	*6th wk.*	*7th wk.*	*8th wk.*	*9th wk.*
I. Detmer's	1	19	42	80	100	120	160	176	216	232
solution	2	21	50	104	140	144	160	200	224	248
(control).	3	21	43	88	108	136	200	224	240	256
	4	20	42	92	124	168	184	192	240	264
	5	21	51	108	132	160	224	208	248	248
	Mean	20·4	45·6	94·4	120·8	145·6	185·6	200·0	233·6	249·6
II. Detmer's	6	25	75	236	392	664	1,336	1,984	2,848	2,976
solution +	7	25	85	256	428	744	1,368	2,120	2,976	3,216
crude nucleic	8	24	70	212	372	616	1,224	1,856	2,840	3,024
acid derivs.	9	22	72	224	360	560	1,256	1,840	2,832	3,256
from 4 grm.	10	25	81	240	356	624	1,312	2,088	2,904	3,200
peat per litre.	Mean	24·2	76·6	233·6	381·6	641·6	1,299·2	1,977·6	2,880·0	3,134·4
III. Detmer's	11	26	79	228	356	520	944	1,288	1,680	1,904
solution +	12	24	69	184	296	464	848	1,128	1,656	1,784
crude nucleic	13	23	68	192	280	472	864	1,208	1,696	1,816
acid derivs.	14	22	61	188	264	424	752	1,096	1,528	1,672
from 2 grm.	15	25	71	208	356	560	1,048	1,512	2,208	2,176
peat per litre.	Mean	24·0	69·6	200·0	310·4	488·0	891·2	1,246·4	1,753·6	1,870·4
IV. Detmer's	16	20	54	136	200	248	464	664	904	968
solution +	17	21	58	156	224	352	536	728	976	1,056
crude nucleic	18	23	60	152	240	336	544	712	904	1,008
acid derivs.	19	19	55	152	200	296	520	640	944	1,024
from 1 grm.	20	22	66	164	224	328	576	672	1,000	1,088
peat per litre.	Mean	21·0	58·6	152·0	217·6	312·0	528·0	683·2	945·6	1,028·8
V. Detmer's	21	26	56	132	212	336	504	680	960	1,048
solution +	22	22	56	128	196	280	472	640	808	896
2 grm. auto-	23	20	52	124	180	280	424	560	776	880
claved *Azoto-*	24	21	53	124	172	264	424	584	688	856
bacter per	25	22	52	128	204	296	504	752	984	1,072
litre.	Mean	22·2	53·8	127·2	192·8	291·2	465·6	643·2	843·2	950·4
VI. Detmer's	26	24	57	140	216	296	440	576	752	848
solution +	27	22	57	116	176	232	424	568	672	728
1 grm. auto-	28	21	50	108	184	264	416	512	664	688
claved *Azoto-*	29	20	50	100	132	192	312	400	544	528
bacter per	30	24	59	132	212	296	464	656	840	960
litre.	Mean	22·2	54·6	119·2	184·0	256·0	411·2	542·4	694·4	750·4
VII. Detmer's	31	22	50	112	164	232	392	488	648	656
solution +	32	22	48	104	180	232	352	456	552	568
0·5 grm. auto-	33	25	60	128	184	264	408	512	648	704
claved *Azoto-*	34	22	54	124	172	256	424	512	656	704
bacter per	35	22	49	108	180	232	376	448	520	576
litre.	Mean	22·6	52·2	115·2	176·0	243·2	390·4	483·2	604·8	641·6

being retained, while the remaining three-fourths were washed and dried in order that their weight might be estimated. At the next weekly counting no reduction was necessary; but at the end of the next week the plants were halved, so that the number remaining represented only one-eighth of the total produce from the original plants. It was found unnecessary to again reduce the number in the dishes before the end of the experiment, when a final estimation of the weight of the plants was made by weighing the contents of two dishes in each series. The numbers of plants in the different series which were obtained at the weekly counting are set out in Table I above, the figures representing the total produce from the original ten plants in each dish, and not those left after the quartering and halving.

A comparison of the increase in number in the different series, brought about by the addition of the various quantities of the added organic matter, shows the following figures:

Crude Nucleic Acid Derivatives.	*Series IV.*		*Series III.*		*Series II.*
Actual increase in number of plants over control set	779		1,621		2,885
Ratio of increase	1	:	2·08	:	3·70
Ratio of quantity of material added	1	:	2	:	4
Autoclaved Azotobacter.	*Series VII.*		*Series VI.*		*Series V.*
Actual increase in number of plants over control set	392		501		701
Ratio of increase	1	:	1·28	:	1·79
Ratio of quantity of material added	1	:	2	:	4

It thus appears that the effect of the crude nucleic acid derivatives is approximately proportional to the amount of this material added, while the effect of the *Azotobacter* is by no means commensurate with the quantity added, though an increase in amount was followed by an increased effect. It is not unlikely, therefore, that the nucleic acid materials do act to a very large extent as actual nutrients, supplying some more or less necessary constituent to the nucleus, while the material of the *Azotobacter* probably functions as a true auximone, enabling the plant to utilize to the best advantage the food materials already supplied.

A comparison of the dry weights of the various sets, estimated at the beginning and end of the experiment, and also on the occasions when the sets were halved or quartered, shows the following result:

TABLE II.

Time.	*Dry Wt. in mg. of* 100 *Plants in Series*						
	I.	*II.*	*III.*	*IV.*	*V.*	*VI.*	*VII.*
At beginning of experiment	10·9	10·9	10·9	10·9	10·9	10·9	10·9
At end of third week	10·8	14·1	13·6	13·4	11·9	12·2	12·3
At end of fifth week	9·9	19·9	20·1	15·5	17·3	16·0	15·8
At end of ninth week	6·5	19·5	19·6	18·9	18·3	18·5	18·9

Not only, therefore, did the added organic matter have the effect of greatly increasing the rate of vegetative multiplication of the plants, but it was also instrumental in increasing the total size and weight of the individual plants, which became gradually larger and more healthy in appearance as the experiment progressed; while those in the control series, which were provided with inorganic nutrients only, actually failed to maintain their original size and vigour, the new shoots successively formed becoming progressively smaller during the course of the experiment. It is noteworthy that the effect of the varying quantities of organic material was not so marked upon the individual size and health of the plants as it was upon their rate of multiplication, for at the conclusion of the experiment the weights of the plants in Series II, III, and IV receiving varying quantities of nucleic acid derivatives were approximately equal, as were those in Series V, VI, and VII receiving correspondingly varying amounts of *Azotobacter* material; and at no time during the experiment was there a very marked difference between the weights of plants receiving different amounts of the same organic material.

It is obvious from the above figures that at least one of the soil organisms, the nitrogen-fixing organism *Azotobacter chroococcum*, has the property of producing something which greatly stimulates plant growth, and Bottomley (7) has shown that another nitrogen-fixing organism, *Bacillus radicicola*, has a similar property. It was therefore decided to test another soil organism not concerned with nitrogen fixation in this way, and *Saccharomyces* (yeast) was selected for the purpose, since yeasts occur fairly commonly in soils, and are known to supply the animal vitamines. Ordinary baker's yeast was obtained, and a weighed portion of this was sterilized in the autoclave in the same way as described above for *Azotobacter*, while another weighed portion was autolysed at a temperature of 35° C. for 72 hours. Both materials were thoroughly shaken up in measured quantities of sterile distilled water in order to ensure a uniform distribution, and a little chloroform was added in each case to prevent bacterial action. When required for use aliquot portions of these liquids containing the requisite weights of the yeast material were measured out, transferred to evaporating basins and freed from chloroform by warming on a water bath, and finally added to the mineral culture solution.

Three series each consisting of five dishes were then arranged as described above. Into each of them were put 200 c.c. of Detmer's solution, and the dishes of Series I, numbered from 1 to 5, received no further addition, but served as controls. To the dishes of Series II, numbered from 6 to 10, was added autolysed yeast at the rate of 0·5 grm. per litre of solution, and to the dishes of Series III, numbered 11 to 15, autoclaved yeast in similar quantity. Ten plants of *Lemna minor* were counted out into each, as before, and three hundred similar plants counted out for an estimation of their dry weight. The solutions were again changed twice weekly and the plants counted once weekly, while the numbers in all the dishes were reduced to one-fourth at the end of the third, fourth, fifth, and sixth weeks, and to one-half at the end of the seventh and eighth weeks, the discarded plants being in all cases washed, dried and weighed, and their dry weights recorded. At the end of the ninth week the experiment was brought to a close. The total numbers of the plants arising from the original ten in each dish at the end of each week are shown in the table below:

TABLE III.

Series.	*Dish No.*	*No. of Plants at End of* *1st wk.*	*2nd wk.*	*3rd wk.*	*4th wk.*	*5th wk.*	*6th wk.*	*7th wk.*	*8th wk.*	*9th wk.*
I. Detmer's solution (control).	1	31	128	500	1,184	3,200	6,400	15,360	25,600	55,296
	2	29	100	376	912	2,752	5,632	14,336	30,720	47,104
	3	31	118	456	1,120	3,072	6,912	14,336	25,600	50,176
	4	30	94	268	624	2,432	5,120	13,824	23,552	60,416
	5	31	104	348	848	2,944	6,144	13,312	24,576	59,392
	Mean	30·4	108·8	389·6	937·6	2,880·0	6,041·6	14,233·5	26,009·6	54,476·8
II. Detmer's solution + 0·5 grm. autolysed yeast per litre.	6	31	155	444	1,152	3,712	11,008	33,280	73,728	247,808
	7	30	128	344	880	2.688	9,728	30,720	68,608	273,408
	8	31	124	504	1,296	4,416	13,312	37,376	98,304	296,960
	9	31	147	492	1,232	4,096	12,544	40,448	95,232	347,136
	10	30	152	552	1,264	4,416	13,056	41,472	102,400	318,464
	Mean	30·6	141·2	467·2	1,164·8	3,865·6	11,929·6	36,659·2	87,654·4	296,755·2
III. Detmer's solution + 0·5 grm. autoclaved yeast per litre.	11	37	149	760	2,192	10,240	32,768	149,504	385,024	1,269,760
	12	31	155	620	1,792	9,152	31,232	148,480	385,024	1,380,352
	13	38	182	736	2,144	9,728	31,744	135,168	319,488	1,105,920
	14	35	128	740	2,160	9,856	31,744	156,672	389,120	1,617,920
	15	35	178	732	2,144	9,664	34,304	158,720	409,600	1.392,640
	Mean	35·2	158·4	717·6	2,086·4	9,728·0	32,358·4	149,708·8	377,651·2	1,353,318·4

A comparison of the dry weights of the plants grown with and without the yeast additions, estimated at intervals during the course and at the end of the experiment, shows the following figures:

TABLE IV.

Time.	*Dry Wt. in mg. of* 100 *Plants in Series* *I.*	*II.*	*III.*
At beginning of experiment	14·5	14·5	14·5
At end of third week	13·7	13·4	15·0
At end of fourth week	13·2	15·2	15·3
At end of fifth week	12·9	16·1	17·0
At end of sixth week	12·3	17·1	17·5
At end of seventh week	11·1	16·5	16·7
At end of eighth week	9·9	17·9	18·6
At end of ninth week	8·1	16·3	16·9

The results obtained thus clearly indicate that the addition of yeast to a culture medium consisting of inorganic ingredients only is as effective as a similar addition of nitrogen-fixing bacteria. Not only did the plants so provided multiply far more rapidly, but they maintained their individual health and vigour, increasing upon their original size, as the increase in their dry weight indicates. In contrast to this effect, the plants growing in solutions containing only inorganic salts failed to multiply so rapidly and also gradually diminished in size, as shown by their loss of weight. It follows, therefore, that some product of the yeast organisms is effective as an auximone, supplying the plant with some necessary material without which it is unable to attain its optimum development, or even maintain its normal health.

A noteworthy fact is the difference in the effect of the autolysed and the autoclaved yeast, the latter producing markedly the greater effect of the two. The difference was noticeable not so much in the individual size of the plants as in their rate of multiplication, for the plants of Series II were to all appearances equal in health and vigour to those in Series III throughout the experiment, and this observation is borne out by their dry weights, which are closely comparable in the two series. It would appear, therefore, that both the autolysed and the autoclaved yeast supply the essential auximone for maintaining complete health, while the autoclaved material contains something in addition which results in a more rapid metabolism and therefore an increased rate of growth and multiplication.

It is well known that yeast is a source of nucleic acid, which occurs in fair quantity in this plant, and it has already been demonstrated that nucleic acid and its derivatives have a marked effect in increasing plant growth. The author (22) has shown that this acid and its derivatives occur very constantly in all organic manurial materials, and that the greater the amount of decomposition which the manure has undergone, and the correspondingly greater the proportion of the original nucleic acid which has become resolved into its purine and pyrimidine derivatives, the greater is the auximonic effect on plant growth of a watery extract of the material. It is probable that in the yeast which was autoclaved for fairly long periods at a temperature of 140° C. the nucleic acid had very largely become resolved into its more simple derivatives. On account of the resistant nature of the nucleic acid, it is more probable that such hydrolysis would have taken place in the autoclaved than in the autolysed yeast; and if this be so, then the greater proportion of the simple derivatives in the autoclaved yeast would obviously be more effective than the smaller proportion in the autolysed yeast, in view of the results quoted above obtained with the varying proportions of nucleic acid derivatives from raw peat.

Having regard to the constant occurrence of nucleic acid and its derivatives in the other materials which have been shown to have an auximonic effect on plant growth, and the similar effect produced by *Azotobacter chroococcum*, it was decided to make an investigation of this organism to determine whether it contained any nucleic acid or derived substance. Details of this investigation are given elsewhere (23), and it is sufficient to state here that a carbohydrate radical, phosphoric acid, purine and pyrimidine bases were all obtained from cultures of the organism, so that whether or not it contains true nucleic acid, at least all the essential constituents of that substance are present. This result has some significance in view of the fact that the marked effect of added organic materials on the size and activity of the nucleus suggests that some nuclear constituent may be involved; and the fact that extracts from soils and from organic manurial materials of all kinds, all of which contain purine and pyrimidine bases, all have the effect of markedly increasing plant growth, supported by the observations of Schreiner, Reed, and Skinner (27) on the action of xanthine and guanine, and those of Schreiner and Skinner (28) on the effect of nucleic acid, hypoxanthine, xanthine, and guanine tends to support the suggestion that some constituent radical or radicals of nucleic acid may be the important factor.

The facts indicated above in connexion with the effect of varying proportions of the crude nucleic acid derivatives suggest that these materials supply a direct nutrient, and do not function as growth-promoting substances in the generally accepted sense of the term. It should be pointed out, however, that the work was carried out with crude extracts, which would contain other substances in addition to the nucleic acid materials, and that it is therefore impossible to assign any particular rôle to one specific substance. The same applies equally to the *Azotobacter* and yeast material used, and even though, in these cases, it is possible that the nucleic acid derivatives may act in this capacity of nutrient materials, there may still be other substances which act as true growth-promoting substances. That such substances are actually present in the products of these organisms becomes obvious from the fact that the increase in growth which results from their addition to inorganic nutrient solutions is beyond all comparison with the actual amount of substance supplied.

In this connexion it is interesting to note that the *Azotobacter* growth, and also a culture of *Bacillus radicicola*, which has been shown to have the effect of increasing plant growth, gave the blue colour-reaction with a preparation of phosphotungstic acid described by Folin and Macullum (12), and which appears to be given by the animal vitamines. Whether this effect is due to the actual purine or pyrimidine materials is uncertain at present, but Williams and Seidell (31) obtained from yeast adenine which acquired, by treatment, vitaminic powers, and which then gave the blue colour with the Folin-Macullum reagent. It appears probable, therefore, that in the case of these micro-organisms, the reaction may be due to the purine bases or to some closely allied substance.

The difficulty of correctly interpreting the results obtained with materials like extracts of peat, *Azotobacter*, and yeast, which contain such a variety of substances, renders it impossible to draw any definite conclusion as to the actual nature of the plant auximones concerned; but the significant fact remains that all the materials which have so far been shown to have this growth-promoting effect on plants also contain, without exception, nucleic acid or its derivatives. The important group of soil bacteria, the nitrogen-fixing organisms, share with yeast the property of elaborating these substances and also the power of increasing plant growth. Whether the nitrogen-fixing organisms are the only group of soil bacteria which carry out this important work of producing plant auximones, is a matter for future investigation; as is also the important question as to whether bacteria in general elaborate the essential radicals of nucleic acid, and, if so, the light which this may throw upon their morphology.

SUMMARY.

1. The effect of varying quantities of nucleic acid derivatives on the growth of *Lemna minor* was investigated, and it was found that the effect obtained was approximately proportional to the quantity of material supplied.

2. Similarly, an examination was made of the effect of varying proportions of a sterilized culture of *Azotobacter*, as a result of which it appears that the effect, though increasing with the quantity of the organism supplied, is not proportional to it.

3. Both autolysed and autoclaved yeast were examined with a view to discovering whether they have any plant growth-promoting power, with the result that both were found to have such, the autoclaved material being more effective than the autolysed.

4. Since the yeast material contains nucleic acid radicals, and a crude extract from peat containing these materials has already been shown to increase the rate of plant growth, an investigation was made of *Azotobacter* to determine whether such substances occur in this nitrogen-fixing organism. Purine and pyrimidine bases, phosphoric acid, and a carbohydrate were all shown to be present. All the necessary radicals for the formation of nucleic acid were thus isolated from *Azotobacter*.

5. The *Azotobacter* growth gave the blue colour with the Folin-Macullum reagent, as also did *Bacillus radicicola*, another nitrogen-fixing organism.

6. The growth-promoting effect of these bacteria may be due to one or more of the nucleic acid radicals present, or to some other substance, but all materials so far investigated and found to have this effect on plant growth also contain these purine and pyrimidine bases.

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A Description of Colletotrichum biologicum, nov. sp., and Observations on the Occurrence of a Saltation in the Species.

BY

H. CHAUDHURI, PH.D. Lond., M.SC. Cal., D.I.C.

(Department of Botany, University of Panjab.)

With Plate XVIII and seven Figures in the Text.

IN early October 1921 a fungus was collected from the dying stalks of potatoes in the garden of the Biologische Reichsanstalt für Forst- und Landwirtschaft at Dahlem-Berlin. The presence of the fungus was shown by a few black sclerotia on the surface of the stem, but just below the epidermis the sclerotia were very abundant. On careful examination under the microscope, the sclerotia were seen to bear black setae; from the sclerotia culture was made on potato extract in agar, and a scanty mycelium rapidly developed, producing innumerable black sclerotia and a few conidia borne irregularly. These conidia were unicellular, and germinated at once, producing similar mycelia and conidia. Single-spore dilutions were made on cover-slips, which were inverted on potato extract agar. As soon as the hyphae from the single-spore culture grew out beyond the cover-slip they produced black sclerotia. Hanging-drop cultures in distilled water were studied; here, too, the spores, after germination, produced sclerotia (as in Text-figs. 2 and 3), but prior to forming sclerotia the germ-tubes invariably produced first thick-walled dark chlamydospores (Text-fig. 1), and these on germinating produced later the sclerotia. The size of the conidia varies considerably; some are $10\mu \times 4\mu$, while others are $20\mu \times 6\mu$, the average being $14\mu \times 5\mu$. The sclerotia are roundish in form, but are somewhat conical at one end, like the fruit bodies of many of the Ascomycetes. A few stiff bristles are always borne by the sclerotia, as already stated. These characters naturally suggest that these structures are immature fruit bodies, but though the fungus has been growing for over seven months in different media, at various temperatures, no perithecia have yet been found; so that

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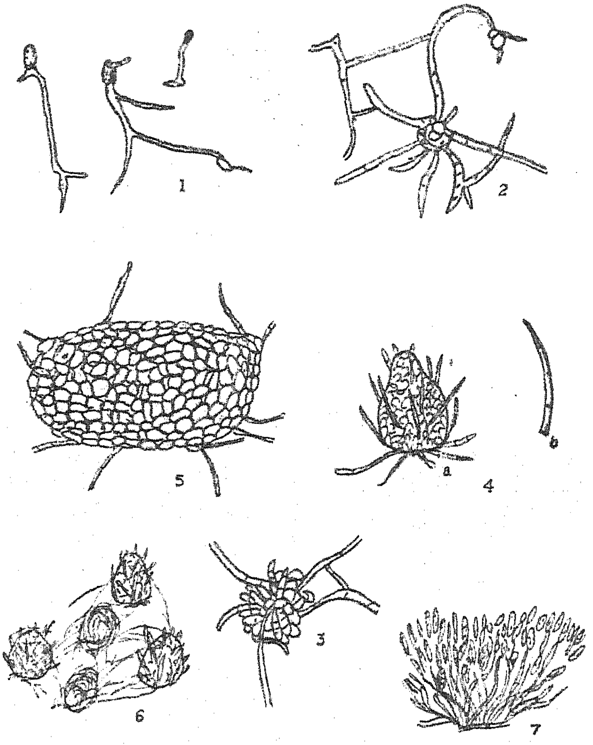
they must be considered merely as sclerotia. The size of the mature sclerotium is 400–500μ × 300–360μ.

For five months this fungus produced only sclerotia and scanty conidia on a weak oatmeal agar, but when placed on strong oatmeal agar it suddenly started producing abundant mycelium and acervuli as well as sclerotia (Text-figs. 6 and 7). The conidia produced in the acervuli were narrower than the conidia borne free on the mycelium, measuring 22μ × 3·5μ on average. Sections were cut of the sclerotia in all stages of development, but no evidence of their ascocarp nature could be obtained.



TEXT-FIGS. 1–7. 1. Germination of a conidium and development of a chlamydospore (*c*). 2. Young sclerotium developing. 3. Sclerotia more advanced. 4. *a*, sclerotia mature; *b*, seta from sclerotium. 5. Section of a sclerotium. 6. Acervulus and sclerotia. 7. Acervulus compressed beneath a cover-slip.

None of the species of *Colletotrichum* hitherto described from Germany has the characters of this fungus. A *Colletotrichum* on potatoes has been reported by P. J. O'Gara from Salt Lake Valley, Utah, which he calls a new species, *Colletotrichum solanicolum*. The characteristics of this fungus given by him (7) much resemble those of the present form, from which, however, it differs in having two distinct types of conidia and much bigger sclerotia. From the similar sclerotia-producing species, viz. *C. tabificum*, Pethybridge, and *C. atramentarium*, Taubenhaus, it is differentiated for the same reasons. There is very little difference between the three species of *Colletotrichum* described by O'Gara, Pethybridge, and Taubenhaus. Taubenhaus (11) says: 'It seems evident that *Phellomyces sclerotiophorus*, Frank, is the same as *Vermicularia atramentaria*, Berk. and Br., and also the same as *Colletotrichum solanicolum*, O'Gara. However, following the rule of priority, the fungus becomes *Colletotrichum atramentaria*, Tauben. Pethybridge (8) expresses doubt as to the reference of *Phellomyces* to *Colletotrichum* rather than to *Spondylocladium*. Pethybridge has considered in this paper *Rhizoctinia tabificum* of Hallier, which formed sclerotia, and which he found consisted of *Verticillium albo-atrum* (which certainly did not form sclerotia) and another fungus which really formed the sclerotia; this last fungus he described as *Colletotrichum tabificum* (Hallier *pro parte*). The descriptions given by Taubenhaus and O'Gara are similar, but comparing these with the description given by Pethybridge we find some difference. In the measurement of the conidiophore O'Gara gives 2–8 μ, whereas Pethybridge gives 20–30 μ. In cultures of the fungus here described the conidiophores are larger in most cases than those given by Pethybridge. It seems probable O'Gara's measurements apply to the segments of the conidiophores, hence the difference between him and Pethybridge. A strong reason against classing the present fungus as *C. tabificum*, Pethybridge, is the complete absence of any amethystine fluorescence, which Pethybridge describes as characteristic of his species.

Accordingly it appears most satisfactory to consider it as a new species, and the name *Colletotrichum biologicum* is suggested.

Colletotrichum biologicum, nov. sp., as grown in culture medium:

Acervulis in gelatina v. agaro *Solani tuberosi* gregariis atris 200–300 μ × 150 μ, interdum coalitis et ampliatis; basidiis cylindricis septatis 30–60 μ × 4–6 μ; conidiis oblongis rectis plerumque guttulatis 22 μ × 5 μ; setulis atris simplicibus rectis acuminatis usque ad 130 μ longis, ad basim 5 μ diam., ad apicem 2 μ diam. Mycelio hyalino septato, hyphis 3–4 μ diam.; conidiis oblongis, numerosis 14 μ × 5 μ; sclerotiis atris 400–500 μ × 300 to 360 μ; chlamydosporis atrofuligineis irregularibus; appressoriis atrofuligineis.

BEHAVIOUR OF THE FUNGUS ON DIFFERENT MEDIA.

This fungus was grown in Coon's synthetic medium (4) in agar, on which, from inoculations from conidia, it prodused a whitish mycelium bearing a few free conidia but at first no sclerotia (Pl. XVIII, Fig. 2 *a*). When inoculated, however, from the sclerotia it produced sclerotia only, and very few conidia (Pl. XVIII, Fig. 2 *b*). Later, in both cases, very minute black bodies somewhat bigger than the chlamydospores were formed.

Further experiments were carried out to determine the behaviour of the fungus towards maltose and asparagin. M/50 maltose agar and M/50 asparagin agar were inoculated with conidia and also with sclerotia. On asparagin agar the conidia produced a pure white mycelium but no sclerotia for a long time, whereas on maltose agar, under the same conditions, very little mycelium but numerous sclerotia were formed (Pl. XVIII, Fig. 3 *a*, &c.). When, however, these media were inoculated from sclerotia instead of conidia, the asparagin agar medium produced a few sclerotia with at first a dark and later a white mycelium instead of the wholly white mycelium produced on asparagin from conidia (Pl. XVIII, Fig. 4 *b*). Inoculation from sclerotia on the maltose agar medium produced a meagre mycelium with innumerable black sclerotia as before. The meagre production of conidia with glucose differs from Stevens's (10) result in his experiments with *Helminthosporium*, and the production of dark-coloured mycelium by asparagin and not by maltose is in opposition to his results; and from the experiments carried on, which will be described under the section of colour production, it will be seen that it is more of a question of temperature than of medium.

SALTATION.

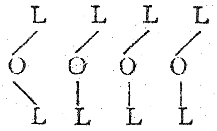
It grew well on potato-mush agar, oatmeal agar, Coon's medium, and various other food materials. This fungus was regularly grown on agar slopes of potato mush and of oatmeal, and transferred to fresh media every fortnight, the old cultures being kept. After four months' culture on oatmeal agar one tube developed a mycelium with pinkish colour, and from that a number of plates on Coon's medium in agar and on oatmeal agar were inoculated. Cultures were also made from the parent tube of the saltant, but no second saltation was found. Inoculations from the saltant were mostly started from single fragments of mycelium. A small portion of the agar medium with the growing zone of the mycelium was taken out with a scalpel, placed and pressed on a glass slide, examined under the microscope, and a single fragment removed for inoculation by means of a platinum needle, all being done under aseptic conditions. From these inoculations there developed parti-coloured cultures—portions of the mycelium being white and bearing normal large sclerotia in concentric rings, while other portions of the cultures in wedgelike form were pinkish and bore small black sclerotia arranged in radiating lines (Pl. XVIII, Fig. 1). From these cultures fresh inoculations were made on Coon's medium in agar, both from the ordinary normal large sclerotia and the smaller ones from the pinkish mycelium. The ordinary large sclerotia when transferred to fresh media produced only the larger ones (Pl. XVIII, Fig. 2). The smaller ones, however, developed a pink mycelium bearing at first only small sclerotia, but later producing whitish mycelium on which large sclerotia developed.

On oatmeal-agar medium the two strains behave as on Coon's medium, but more mycelial growth with free conidia is produced and many acervuli are developed. On potato extract, however, the two strains cannot be differentiated; when the smaller ones are inoculated into potato-extract agar or potato-mush agar, neither the coloured hyphae nor smaller sclerotia in radial rows are produced, but only large sclerotia in concentric rings. When, however, several generations of the new strain have been grown in Coon's medium in agar or on oatmeal agar—in which coloured mycelium and radiating sclerotia are always produced—and it is then transferred to potato extract agar, only a few sclerotia in radiating lines are produced on a very faintly coloured mycelium. Continued growth, however, on potato extract in agar for a couple of new generations leads to the complete loss of colour and the production of smaller radiating sclerotia. The behaviour of the saltant is never perpetuated in potato-extract agar; after growing the saltant for over twenty generations in Coon's medium, when it was put back on potato-mush agar and carried through a couple of generations all traces of the saltant were lost for good. Mutation in *Colletotrichum gleosporioides* has been mentioned by Burger (3). In his plate cultures of certain isolations of *C. gleosporioides* he found parts of the mycelium usually in wedge-shaped or fan-like areas showing a different colour from that of the rest of the growth. These variations were continued when transfers were made from the modified areas. Burger's *Colletotrichum* constantly gave off new types under natural as well as artificial conditions, and as these variations were more or less permanent they have been regarded as mutations. Mention of constantly mutating species, like Burger's, has been made by various workers, viz. Egerton, Dastur, Stevens, and others. Egerton (6), working with *Glomerella rufomaculans*, found permanent variations in a number of cases which may be looked upon as saltation. Dastur (5) found his original cultures of *Gleosporium piperatum* breaking suddenly into newer strains, and also that the mutation was a common phenomenon only in strains that were recently derived from the perithecia, for he observed no sudden variations from conidial strain. Stevens (10), working with various species of *Helminthosporium*, found that certain strains of a colony growing on an agar plate differed more or less from the rest of the colony, and when fresh transfers were made to new agar plates these differences were usually maintained on succeeding agar plates; and this phenomenon, which Stevens called saltation, was of rather common occurrence. Stevens also lends support to Dastur's view of recent perithecial origin favouring variations. The only mutation that occurred in this case was in the fifteenth generation from the original culture after a period of four months. Reviewing mutations which occur less frequently, the work of Shear and Wood (9) with *Glomerella* is of interest. They found important variations or mutation suddenly occurring in the fourth generation, and thus was transmitted through a number of generations, but other variations also occurred which have been less permanent and have been regarded as fluctuations. In a recent paper Blochwitz (1) reports an interesting case of mutation in *Aspergillus versicolor*, which has usually a green colour. On making gelatin cultures he found in one generation three plates which produced a perfectly blue coloration, and cultures from these plates gave the blue pure form through over forty generations. Though the old cultures had been kept and subcultures were always made, no other case of blue coloration had appeared. Discussing the colour production, he says it is due to two things: (1) colour in the membrane, and (2) colour in the cell contents. In his mutation there was no coloration in the membranes of the conidia or mycelium. The disappearance of colour he ascribes to old age or due to long asexual reproduction. But in both cases the changes are very gradual, whereas in his three plates the changes were sudden and have been regarded as mutations.

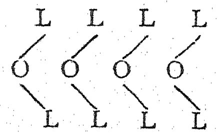
Dealing with cases in which saltation or mutation has occurred only once, as in the present fungus, Brierley (2), working with *Botrytis cinerea*, which normally produces only black sclerotia, found in one of his single-spore inoculations in a Petri dish a mycelium which formed only pale sclerotia, and this new form reproduced itself in culture. In the present case, though the fungus has been grown for nearly two years, no second change has occurred. The first change occurred in a race that had been under culture for more than four months. As the change is permanent and shows its distinctive characteristics even when grown with the original under the same conditions, it has been termed a saltant. *Colletotrichum biologicum* has thus given rise suddenly to a saltant or new strain, the original being constant in character and never producing small sclerotia, while the new strain can produce both large and small sclerotia according to external conditions, i.e. according to the nature of the medium on which it is grown.

The following table shows the result of putting the saltant on potato-mush agar from the first up to the twentieth generation. Here O stands for the old normal strain, and ɵ stands for the newer strain that has arisen by mutation from the older one; L for large sclerotia and S for smaller sclerotia.

Potato-extract agar



Oatmeal agar and Coon's medium agar



Oatmeal agar at time of saltation	L O L	L O L	L Θ S	L Θ S	L Θ S
Coon's medium agar inoculated with Θ	L Θ S	L Θ S	L Θ S	L Θ S	
Potato-extract agar inoculated with Θ of the first generation	L Θ S	L O S	L O S		
Potato-extract agar inoculated with Θ of the fifth or sixth generation or Θ of the twentieth generation	L Θ S	L Θ L	L O L	L O L	

During the whole course of the experiment subcultures were made fortnightly and the older parent cultures were preserved. When the new strain had appeared a number of subcultures were made from the parent cultures, but in no case did the second newer strain develop. The newer strain had evidently arisen by a single mutation. It is absolutely necessary in connexion with the work on mutation to see that the cultures are started either from a single spore or conidium or a small portion of mycelium. In the present work cultures both from conidia and single fragments of mycelium were made. The new strain differs from the old one in producing coloured hyphae and also smaller sclerotia; these two strains were grown, like the original strain, on Coon's medium in agar, on M/50 maltose agar, and M/50 asparagin agar, to determine if they behaved differently. Inoculation from either into asparagin agar produces coloured hyphae and some sclerotia arranged in concentric rings (Pl. XVIII, Fig. 4 *a*), but from the smaller ones sclerotia arranged both in concentric and radiating lines are produced (Pl. XVIII, Fig. 4 *c*).

PRODUCTION OF COLOUR AND ITS RELATION TO TEMPERATURE.

The new strain produces a very bright pinkish colour at low temperature, and a black colour at higher temperatures. When the black mycelial growth developed at a higher temperature is inoculated into a fresh medium which is incubated at a low temperature the pink colour characteristic of the low temperature is produced. Similarly a black mycelium is produced if inoculated from a pink of lower temperature, but incubated at a higher temperature. Even when a culture which is growing at a high temperature, and producing the black mycelium, is placed at a low temperature, the new growth is pinkish in colour, the black mycelium remaining unchanged. When, however, a pink mycelium developed at a low temperature is transferred to a higher temperature, all the mycelium becomes black, i.e. the newly produced and the original pink mycelium. The pink colour becomes fainter above 20° C., and at 22·5° C. it becomes almost dark; at 25° C. or over it is perfectly black.

GROWTH AND TEMPERATURE.

These two strains were grown at various temperatures, viz. 12°, 14°, 16°, 18°, 20°, 22·5°, 25°, 27°, 30°, and 32°, in Coon's normal medium, to determine if there was any difference in their amount of mycelial 'spread'. Besides inoculations with two kinds of sclerotia, inoculations from conidia were also made. As will be seen from the following table, the amount of 'spread' was always the same. The following table gives the measurement of the diameter of colonies grown in Petri dishes for seven days at different temperatures:

Temp. C.	*Parent.*	*Saltant.*	*Large.*
12°	15 mm.	15 mm.	15 mm.
14°	20 ,,	20 ,,	20 ,,
16°	25 ,,	25 ,,	25 ,,
18°	28 ,,	28 ,,	28 ,,
20°	35 ,,	35 ,,	35 ,,
22·5°	40 ,,	40 ,,	40 ,,
25°	46 ,,	46 ,,	46 ,,
27°	52 ,,	52 ,,	52 ,,
30°	47 ,,	47 ,,	47 ,,
32°	nil.	nil.	nil.

SUMMARY.

1. *Colletotrichum biologicum*, nov. sp., collected in Germany in early October 1921 from dying stalks of potatoes, is described.
2. Only innumerable sclerotia with black setae were found on it in nature.
3. The cultural characteristics of this fungus were studied on different media and under various environmental conditions.
4. On potato-mush agar, Coon's medium, and on weak oatmeal agar it produced large sclerotia in concentric rings, scanty mycelia and conidia, but on strong oatmeal agar it produced abundant mycelium with acervuli as well as sclerotia.
5. Asparagin increased mycelial growth, whereas maltose increased sclerotia production.

6. After four months' culture in the laboratory (16 generations) saltation occurred, for it suddenly produced a new strain with a coloured mycelium bearing not only the large sclerotia in concentric rings of the parent form, but also small sclerotia in radiating lines.

7. Though the original culture has been continued on oatmeal agar, the medium in which it mutated, and also on various other media, for nearly two years, no second change has appeared.

8. The saltation is permanent and keeps its characteristics on Coon's medium in agar, and on oatmeal agar. When grown, however, on potato-mush agar it soon loses completely its distinctive characteristics and becomes the same as the original. The loss is permanent, for when put back to oatmeal agar or Coon's medium it fails to show the characteristics of the saltant.

9. There is no difference between the two forms in relation between growth and the effect of temperature. The optimum for both strains was 27° C.

10. The production of different colours by the saltant has been found to be entirely dependent on temperature.

The greater part of the work was done in the laboratory of the Department of Plant Physiology and Plant Pathology of the Imperial College of Science and Technology, London, during 1921-2. The paper has been finally completed in the Botany Laboratory of the Panjab University. The writer is indebted to various persons for their kind help. His best thanks are due to Professor V. H. Blackman, F.R.S., for his constant interest and helpful suggestions. He is also indebted to Dr. E. J. Butler, of the Imperial Mycological Bureau, Kew, and to Mr. Ramsbottom, of the Natural History Museum, South Kensington, for their help in the determination of the fungus. He is further indebted to Mr. Ramsbottom for valuable assistance with the Latin description, and to Dr. Horne and Dr. Brown for their many suggestions and criticisms, and to Mr. Tooley for taking the photographs of the culture plates.

LAHORE.
October 1923.

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EXPLANATION OF PLATE XVIII.

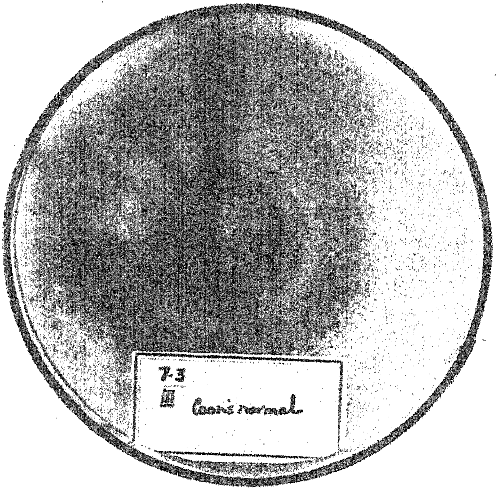
Illustrating Dr. H. Chaudhuri's paper on *Colletotrichum biologicum.*

Fig. 1. Plate-culture showing 'sectorial' growth, the new strain appearing as fan-like dark masses.

Fig. 2 *a*. Conidial inoculation on Coon's normal agar; white mycelium only produced. Fig. 2 *b*. Sclerotial inoculation on Coon's normal agar; innumerable sclerotia and scanty mycelium produced.

Fig. 3 *a*. Conidial inoculation on asparagin agar. Fig. 3 *b*. The same on oatmeal agar; the white dots in the centre are acervuli. Fig. 3 *c*. Conidial inoculation on maltose agar.

Fig. 4 *a*. Culture on maltose agar with large sclerotia only. Fig. 4 *b*. Culture on asparagin agar producing both large and small sclerotia. Fig. 4 *c*. Culture on maltose agar producing both large and small sclerotia.



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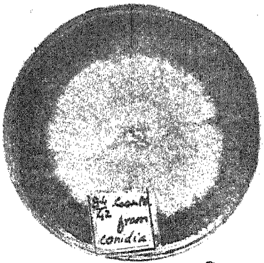

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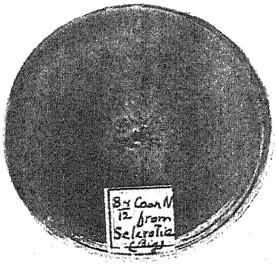

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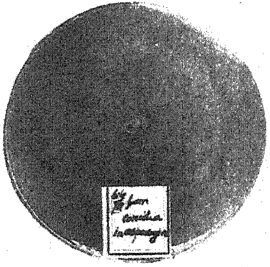

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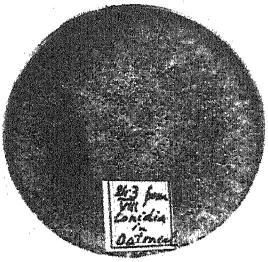

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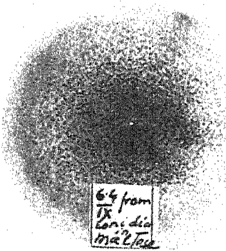

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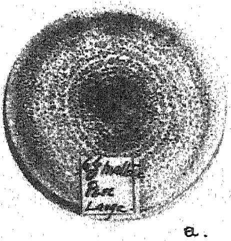

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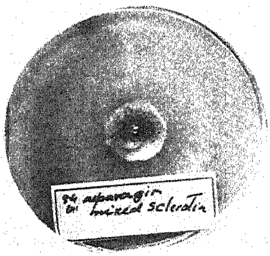

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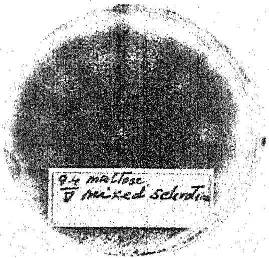

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Effect of Methyl and Ethyl Alcohol on the Growth of Barley Plants.

BY

AMAR NATH PURI,

Rothamsted Experimental Station.

With three Figures in the Text.

I.

INTRODUCTION.

ETHYL alcohol is known to be highly toxic to the protoplasm of the cells of the *Elodea* leaf (1). The effect is believed to be due in the main to increased permeability, which results in the exosmosis of the cell contents. Seifriz (2) has studied the antagonistic effect of salts on the toxicity of alcohol. The results show that, whereas calcium chloride never opposes the ill effects of ethyl alcohol on protoplasm, sodium chloride does act as an antagonizer, though not always completely. On the other hand, a combination of sodium chloride and calcium chloride acts unfailingly as an almost perfect inhibitor of the toxic influence of 10 per cent. alcohol.

The toxicity of alcohol when applied to the roots of plants has apparently not been studied. The object of this work was to see how far methyl and ethyl alcohol act as stimulants or poisons to barley plants, when added to the food solution in water-cultures.

EXPERIMENTAL.

Seeds were graded between 0·05 and 0·06 grm.

The following concentrations of alcohol in the food solution were employed:

Series.	*Ratio of alcohol to water.*
1.	1 : 200
2.	1 : 1,000
3.	1 : 5,000
4.	1 : 25,000
5.	1 : 125,000
6.	Control

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The amounts of nutrient materials in the food solution were as follows:

KNO_3	1	grm.
$MgSO_4$	0·5	,,
KH_2PO_4	0·5	,,
$NaCl$	0·5	,,
$CaSO_4$	0·5	,,
$FeCl_3$	0·04	,,

To make up one litre.

The water used in these experiments was distilled in a special still provided with a silver condenser (3). Each bottle contained about 600 c.c. of food solution, made up by adding 300 c.c. of nutrient solution of twice the strength given above, and 300 c.c. of alcohol solution, containing the necessary amount of alcohol. The experiments were conducted in triplicate, and the results with the two alcohols are presented separately.

ETHYL ALCOHOL.

Twelve days after placing the seedlings in the culture solutions, plants in Series 1 (conc. 1:200) appeared smaller than the rest; all others were normal and apparently of the same size, &c. Three weeks after, the plants in concentrations 1:200 and 1:1,000 were practically dead and were thrown away. The growth in concentration 1:5,000 was distinctly impaired. In concentrations 1:25,000 and 1:125,000, except a faint suggestion that the roots were slightly smaller than those of the control plants, no ill effects were visible. During the next five weeks, plants supplied with alcohol, though healthy and normal, remained slightly shorter than the control. In another two weeks, however, all these plants made up the difference and grew in height equal to the control. After a further period of two weeks, these plants looked bigger than the control. It appeared, however, that this sudden growth was entirely confined to ear shoots, other leaves ceasing to grow, and showing signs of dying off. The ears looked just as good as, or perhaps better than those of the control plants. It seemed as if all the energy was being directed towards the production of ear shoots. It was noticed that in Series 3 and 4 (conc. 1:5,000 and 1:25,000) the roots were rather slimy at the tips.

The above results suggest that, if alcohol be given to plants when they have grown up to a certain extent, it might have a stimulating effect. This point was tested and the results are recorded in Part II of this investigation.

The plants were taken out after twelve weeks, roots separated from shoots, and dried. Table I gives the average length of the roots and shoots, and their dry weights.

TABLE I.

Series.	*Conc. of Alcohol.*	*Length.*		*Dry weight (grm.).*	
		Root. in.	*Shoot.* in.	*Root.*	*Shoot.*
1.	1 : 200	—	—	—	—
2.	1 : 1,000	—	—	—	—
3.	1 : 5,000	6·5	21·0	2·30	8·72
4.	1 : 25,000	7·3	15·0	2·86	9·56
5.	1 : 125,000	6·0	17·0	3·40	11·82
6.	Control	6·3	15·3	2·55	11·15

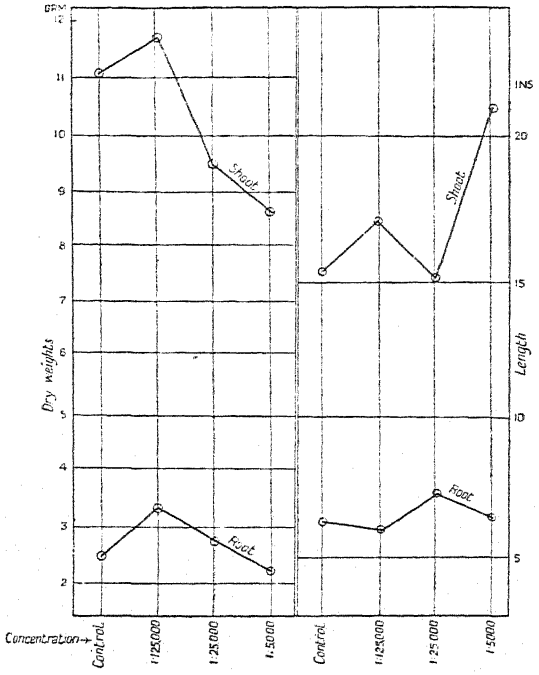

FIG. 1. Average dry weights and lengths of barley plants grown in nutrient solutions with different concentrations of ethyl alcohol.

The above results are shown graphically in Fig. 1. There is an indication of stimulation in very small concentrations. The lengths of the plants do not show any marked difference in the roots. The extra length of the shoots in Series 3 (conc. 1 : 5,000) emphasizes the fact, pointed out before, that the lower toxic doses of ethyl alcohol tend to push forward the development of ear shoots at the expense of the vegetative leaves, so that, although the average length of plants was greater than the control, their dry weights were smaller. It will be seen that alcohol in concentrations 1 : 1,000 and higher is fatal to barley plants, as is apparent from the death of all the plants in Series 1 and 2 within three weeks. It was also noticed that when plants were killed by these high concentrations the dead roots and the surface of the solution were infested with copious growth of moulds.

METHYL ALCOHOL.

A set of experiments, similar to the above, was carried out with methyl alcohol, the strength of solutions and other conditions being the same as in the case of ethyl alcohol. The average length of roots and shoots, as well as their dry weights, are recorded in Table II and shown graphically in Fig. 2.

TABLE II.

Series.	*Conc. of Alcohol.*	*Length (inches).*		*Dry weight (grm.).*	
		Root.	*Shoot.*	*Root.*	*Shoot.*
1.	1 : 200	3·8	13·2	1·60	7·20
2.	1 : 1,000	4·5	13·6	1·56	6·20
3.	1 : 5,000	6·0	15·0	2·16	8·50
4.	1 : 25,000	7·3	17·8	2·80	10·50
5.	1 : 125,000	6·3	18·6	2·83	10·26
6.	Control	6·3	17·6	2·61	10·20

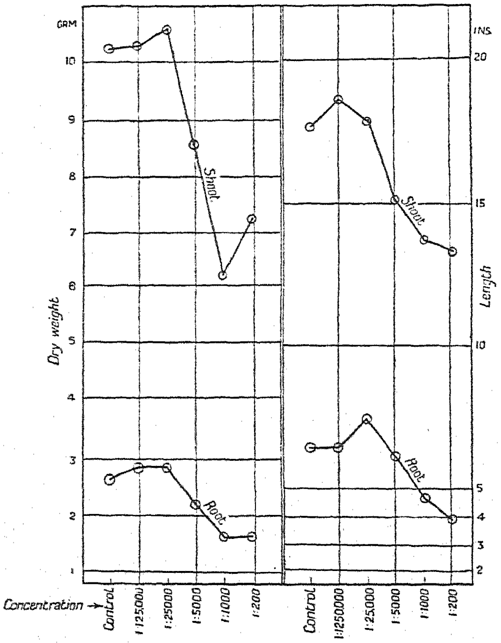

FIG. 2. Average dry weights and lengths of barley plants grown in nutrient solutions with different concentrations of methyl alcohol.

The essential differences between the effect of methyl and ethyl alcohol were:

(1) Plants grew in the presence of methyl alcohol concentrations up to 1 : 200, though in higher concentration the growth was stunted; while in

the case of ethyl alcohol concentration of 1 : 1,000 was found to be fatal. This would seem to show that ethyl alcohol is more toxic than methyl alcohol.

This result is in agreement with that of Vandevelde (4), who studied the toxic effects of various alcohols on the cells of the red onion, and found that the poisonous nature of the monohydric alcohols increases with the molecular weight.

(2) In higher concentrations, ethyl alcohol favoured the growth of ear shoots and the suppression of vegetative leaves, whereas methyl alcohol favoured the growth of leaves and not the ear shoots. This difference in the behaviour of the two alcohols is extremely interesting and difficult to account for.

The difference between the physico-chemical properties of the two alcohols is so small that any explanation based on their effect on the permeability of cell-walls would be difficult to picture; cf. Seifriz (2). It may be noted that there seems to be no other parallel instance of two substances, so much alike in chemical constitution as the alcohols, showing a radical difference not only in the degree of toxicity but its type.

II.

EFFECT OF ADDING ETHYL ALCOHOL TO BARLEY PLANTS AT DIFFERENT STAGES OF GROWTH.

It was pointed out in the earlier part that barley plants supplied with alcohol showed a very rapid growth after a certain time. In order to elucidate further information with regard to this observation three sets of experiments consisting of forty plants each were employed.

Set A. Alcohol added from the beginning.
Set B. Alcohol added after three weeks of growth.
Set C. Alcohol added after six weeks of growth.

The following concentrations of ethyl alcohol were employed:

1. 1 : 200
2. 1 : 1,000
3. 1 : 5,000
4. 1 : 25,000
5. 1 : 125,000
6. 1 : 250,000
7. 1 : 500,000
8. Control.

The experimental method and technique were the same as in the earlier work. The general conclusions in Set A, where alcohol was added from the beginning, were the same as in the previous experiments, except that plants in concentrations 1 : 1,000, although they only developed a single weakly shoot with little leaf growth, managed to struggle through, while previously they had all died. The suggestion that alcohol might act as a stimulant when given to plants at advanced stages of growth was not confirmed. It was observed, however, that plants can partly resist the poisonous action of alcohol when they are grown up to a certain extent. It is interesting to compare this result with that of Vandevelde (4), who found that older cells withstand the action of the alcohols better than the younger. Brenchley (5), who studied the toxic effect of phenols on barley plants, also concluded that, when once growth is fairly under way and vigorous, the plant can cope with a strength of poison which is most deleterious at an earlier stage of development.

Plants were kept in the culture solution for ten weeks, after which they were taken out, dried, and weighed. The mean dry weights of the various plants, along with the probable error, are given in Table III.

TABLE III.

Set A. Alcohol added from the beginning.

	Conc. of Alcohol.	*Root.*		*Shoot.*		*Root. Shoot.*	
1.	1 : 200	—	—	—	—	—	—
2.	1 : 1,000	0·33	± 0·027	0·91	± 0·087	1·24	± 0·114
3.	1 : 5,000	0·88	0·131	3·37	0·369	4·25	0·50
4.	1 : 25,000	0·60	0·096	3·57	0·422	4·17	0·518
5.	1 : 125,000	1.05	0·092	5·65	0·213	6·70	0·285
6.	1 : 250,000	1·03	0·081	5·71	0·284	6·74	0·365
7.	1 : 500,000	1·11	0·109	5·94	0·328	7·05	0·437
8.	Control	1·03	0·081	5·53	0·314	6·56	0·395

Set B. Alcohol added after three weeks.

	Conc. of Alcohol.	*Root.*		*Shoot.*		*Root. Shoot.*	
1.	1 : 200	—	—	—	—	—	—
2.	1 : 1,000	0·60	± 0·070	2·28	± 0·194	2·88	± 0·264
3.	1 : 5,000	1·21	0·101	5·10	0·178	6·31	0·279
4.	1 : 25,000	1·08	0·060	5·28	0·208	6·36	0·268
5.	1 : 125,000	1·32	0·105	5.22	0·246	6·54	0·351
6.	1 : 250,000	0·92	0·066	4·91	0·165	5·83	0·231
7.	1 : 500,000	1·00	0·062	5·02	0·163	6·02	0·225
8.	Control	1·32	0·069	6·01	0·359	7·33	0·428

Set C. Alcohol added after six weeks.

	Conc. of Alcohol.	*Root.*		*Shoot.*		*Root. Shoot.*	
1.	1 : 200	0·99	± 0·051	4·31	± 0·238	5·30	± 0·289
2.	1 : 1,000	1·28	0·061	5·36	0·189	6·64	0·250
3.	1 : 5,000	1·11	0·083	5·56	0·096	6·67	0·179
4.	1 : 25,000	1·24	0·083	5·75	0·232	6·99	0·315
5.	1 : 125,000	1·07	0·002	5·14	0·133	6·21	0·135
6.	1 : 250,000	1·26	0·034	6.05	0·071	7·31	0·075
7.	1 : 500,000	1·37	0·091	6·09	0·215	7·46	0·306
8.	Control	1·22	0·130	6·51	0·093	7·73	0·223

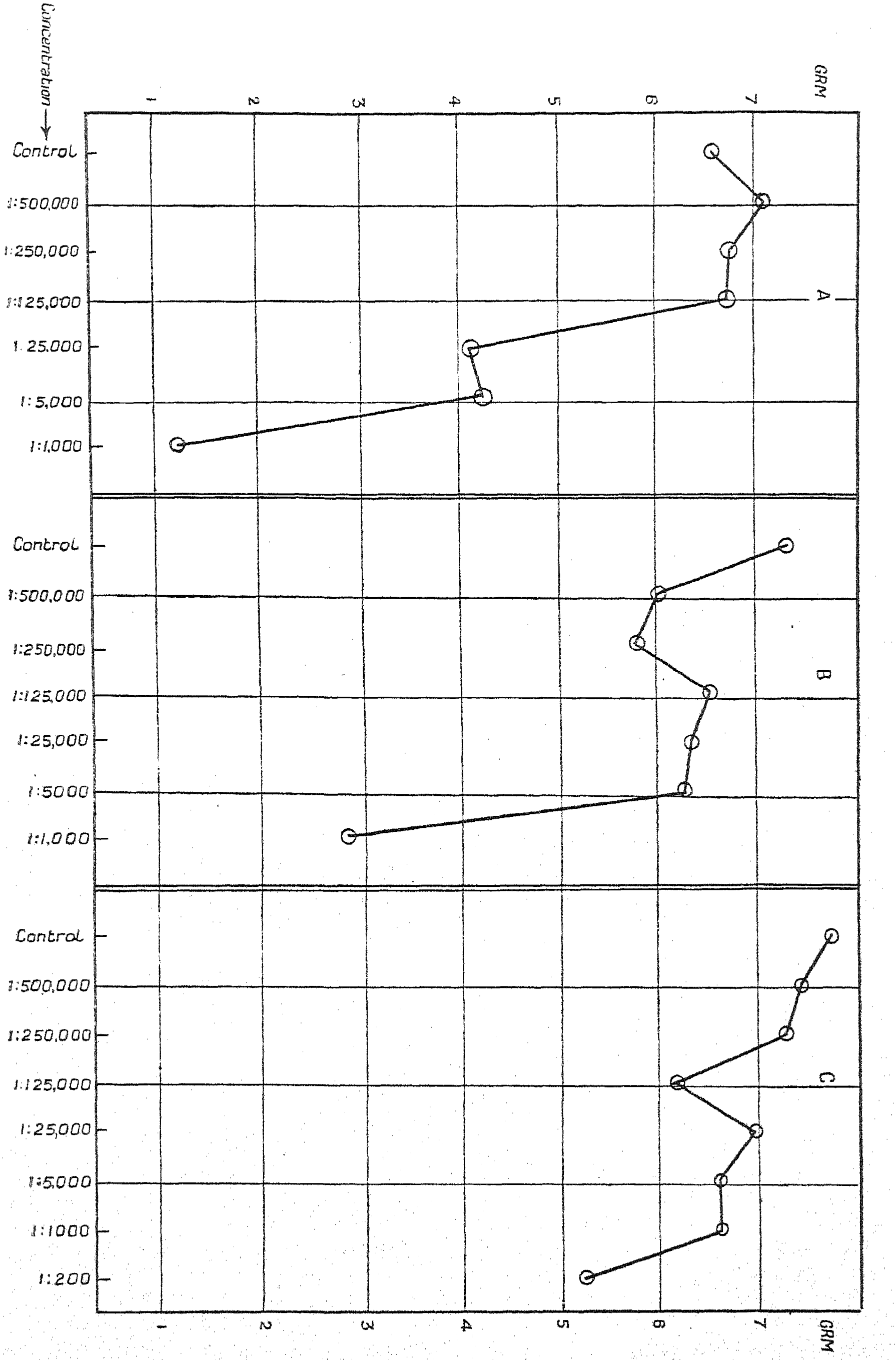

FIG. 3. Average dry weights of three series of barley plants grown in nutrient solutions with different concentrations of ethyl alcohol added at different stages of growth. A. Alcohol added at the beginning. B. Alcohol added after three weeks. C. Alcohol added after six weeks.

The above results are graphically represented in Fig. 3 and bring out very clearly the conclusion that the toxic effect of alcohol is appreciably reduced when it is administered at a later stage of growth. It will be seen that in Set A the poisonous action appeared in concentrations 1 : 25,000 and higher, while in Set B it is noticeable at concentrations 1 : 1,000 and higher; whereas in Set C it is observed only in concentration 1 : 200 to an appreciable extent. It may be concluded from the foregoing results that when the plant is developed to a certain extent it can resist the action of poison much better than in the earlier stages of growth. In studying the toxic effect of substances, therefore, it is necessary to pay due regard to the age of the plant when the poison is first administered.

SUMMARY.

1. Ethyl alcohol is more toxic to barley plants than is methyl alcohol.
2. The difference in the toxicity of the two alcohols is not only one of degree, but of kind; ethyl alcohol favours the growth of ear shoots and the suppression of vegetative leaves, while methyl alcohol favours the growth of leaves and not that of the ear shoots.
3. Plants in their later stages of growth can withstand the toxic action of ethyl alcohol much better than earlier in life.

The work here described was carried out under the direction of Dr. W. E. Brenchley, and it is my pleasant duty to record my thanks to her for the facilities provided me, and for many valuable suggestions. My acknowledgements are also due to the Punjab Drainage Board for the grant of the scholarship which gave me the opportunity of undertaking this work.

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The Antagonism between Dyes and Inorganic Salts in their Absorption by Storage Tissue.

BY

CHARLES E. T. MANN, M.Sc.

With eight Figures in the Text.

THE absorption of dyes by living plant tissues was first systematically investigated by Pfeffer (12), who found that, when various dyes were presented in dilute solution to living tissues of a large number of plants, the dye was absorbed until the concentration of dye within a living cell was far higher than the concentration of the dye in the external solution. It was suggested that this phenomenon might be explained on the assumption that the dye formed a non-diffusible compound with some substance present in the cell sap. The investigations of Pfeffer were mainly confined to the absorption of the dyes from solutions of single substances, but his methods of experiment have more recently come to the fore in investigations of the behaviour of living tissues towards solutions containing more than one substance in which the tissues are immersed.

That the presence of a second dissolved substance reduces the harmful action of a soluble toxin has for long been an established fact. In this connexion may be mentioned the work of Loeb and Gies (7), who showed that the addition of a second salt, the chloride of Ca or Mg, reduced the poisonous action of NaCl on the development of the eggs of the marine fish *Fundulus*.

Further work on this subject by Osterhout (10), mainly concerned with the retardation of the absorption of one salt in the presence of a second salt, may also be mentioned.[1]

The same phenomenon also exists in solutions containing organic substances and inorganic salts: von Eisler and von Portheim (3) demonstrated that the toxic action of quinine was reduced by the addition of salts of

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[1] To speak of the absorption of salts as such is hardly correct, for it has been observed that the ions of a salt may be absorbed at different rates. Further reference to this phenomenon is made on p. 760.

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potassium, calcium, magnesium, or aluminium to the toxic solution. Szücs (19) also, working with quinine hydrochloride, found that the nitrates of potassium, calcium, and aluminium 'antagonized' the entry of the organic substance into living cells of a species of *Spirogyra*, the degree of antagonism being greatest in the case of aluminium nitrate. The experimental procedure employed by Szücs in this work was extended by him (20) to an investigation of the intake of aniline dyes in the presence of electrolytes. The time taken by a filament of *Spirogyra* to reach a standard coloration when immersed in a solution containing a salt and dye in definite concentrations was observed. From the results obtained, Szücs concluded that the 'antagonistic' action of an electrolyte towards a dye depends on the concentration and the valency of the kation. The relationship holding between the time taken to reach a standard coloration and the concentration of the electrolyte present approximates to the adsorption equation. This fact is put forward as an argument in favour of the theory that the intake of the dye is prevented owing to the adsorption of the electrolyte by the protoplasm.

That antagonism is due to a mutual hindrance to the entrance of substances into the cell receives evidence in support from the experiments of Szücs, as the solution containing salt and dye undergoes no change, no reaction occurring outside the tissue.

Using various algae and *Elodea*, a further investigation of the phenomenon was made by Endler (4). In these experiments only the soluble dyes, methylene blue and neutral red, were employed, and only the total amount of the dye absorbed by the living tissue in solutions of several salts was estimated. It was found that intake of the dye increased at first with concentration of the electrolyte present, but decreased on further increase in the salt concentration.

So far in all investigations little account has been taken of the rate of absorption of the salt from a mixed solution of an electrolyte and a dye. Usually the relative concentration of salt and dye are such that the molecular concentration of the salt is very much greater than that of the dye, and little alteration would be expected in the rate at which the salt enters the tissues in the presence or absence of the dye.

Actual quantitative data on this subject of antagonism between an electrolyte and a dye are few, most investigations having been qualitative. In the following investigation an attempt has been made to obtain actual quantitative data of the intake of methylene blue, neutral red, and orange G when presented to living tissues in the presence of the chlorides of ammonium, magnesium, aluminium, and lanthanum. Throughout the investigation the initial quantity of dye used was kept constant, and the salts were employed in varying concentrations.

The work was undertaken at the suggestion of Professor W. Stiles, and

was carried out in the Botanical Laboratory of University College, Reading. The cost of apparatus employed was partly defrayed from a grant made to Professor Stiles by the Government Grant Committee of the Royal Society, to whom acknowledgements are therefore due.

METHOD.

The essential features of the method employed throughout the investigation were as follows: Discs of mangold tissue, 2 cm. in diameter and 0·1 cm. thick, were immersed in a solution of the dye containing a salt in definite concentration. At recorded intervals samples were drawn from the solution and analysed colorimetrically, and also, in stated cases, chemically. From the results of analysis the quantities of dye and the salt taken up by the tissue were computed for a stated time. The necessary adjustment of the ratio holding between the volume of the solution and the weight of tissue employed was made by removing discs of the tissue at the same time that samples of the solution were withdrawn. The tissue used in the following experiments was the white parenchymatous storage tissue of *Beta vulgaris*, var. Sutton's Yellow Globe. The material was grown on a heavy loam soil, and mangolds of approximately the same weight were used throughout. Mangold has not previously been used for this kind of work, but it possesses certain advantages over some other storage tissues. On account of size, mangold roots yield very uniform sections, and a large number of these, sufficient for a whole series of comparative experiments, may be obtained from a single root. A second distinct advantage is that no pigment is present which is likely to obscure colorimetric estimations carried out on solutions in which the tissue has been immersed. The fact that even after three days' immersion in a solution containing orange G and ammonium chloride the discs of mangold were quite fresh and exhibited a slight increase in weight is sufficient indication that the vitality of the tissue is not impaired by a period of immersion lasting twenty-four hours, the period over which the bulk of the experiments were conducted.

In preparing the discs of mangold tissue, it was found necessary to adopt the following procedure: The mangold was washed and cut transversely into slices, so that usually six slices of approximately 10 cm. in diameter and 2 cm. in thickness were obtained. The outer tissues were removed and the material placed in distilled water in a dark room. After a period of twelve hours, cylinders of tissue were cut from the prepared slices by means of a well-sharpened cork-borer, and from these cylinders the discs were cut on a hand microtome. After a further period of soaking in distilled water, lasting for approximately twelve hours, the prepared discs were washed in several changes of conductivity water, and lightly dried in sets of forty between sheets of smooth white blotting-paper. A single set of forty discs was employed in each experiment, and usually approximately 300 discs were prepared in a single operation as described above. This method of preparation of discs of storage tissue is a modification of the method elaborated by Stiles and Jørgensen (16) and Stiles and Kidd (18) in investigations of the absorption of salts by plant tissues.

Though water is still being absorbed by discs of tissue after a period of twenty-four hours' immersion, further increase in weight is so small as to be practically negligible over the period of an absorption experiment. As the weights of sets of forty discs varied slightly, experiments were conducted in duplicate, and in many instances in triplicate. The average weight of a single set of forty discs was 12·34 grm., calculated on twenty-five weighings. The probable error of this mean, calculated on the expression $\text{P.E.} = \frac{2}{3}\sqrt{\frac{\Sigma d^2}{n\,(n-1)}}$, where Σd^2 is the sum of the squares of differences from the mean, and n the number of readings, was 0·0788.

A set of discs, prepared and lightly dried as described above, was weighed and quickly transferred to a wide-mouthed glass jar of approximately 400 c.c. capacity, fitted with a well-ground stopper and containing 200 c.c. of the experimental solution. The jar was placed in a constant temperature bath, and samples of the solution were withdrawn at intervals of from one to twenty-four hours. On removal of a portion of the solution it was also necessary to remove a corresponding proportion of the tissue immersed, and this was performed in the following way: The disc to be removed was impaled on the curved point of a finely-drawn glass rod and raised a little above the surface of the solution in the jar, where it was held in forceps. To remove the small quantity of the solution the disc of tissue was gently flicked with the glass removing-rod. This treatment was found to be rapid and effective, and introduced no serious source of error. Where only a colorimetric estimation was required, usually 10 c.c. of the solution and two discs of tissue were removed.

In this investigation the dyes used were methylene blue, neutral red, and orange G, and in each instance a stock solution containing 1 grm. per litre was prepared. The salts employed were the chlorides of ammonium, magnesium, and aluminium; a normal solution of each was prepared and standardized against silver nitrate. A few experiments were also performed with lanthanum chloride. From these stock solutions the necessary experimental solutions containing definite concentrations of salt and dye were prepared as required.

Two methods of estimating the salt concentration of a solution containing salt and dye were employed in the case of ammonium chloride. In the first method, employed when methylene blue was present, it was found possible to estimate the salt as chloride in the usual way by means of silver nitrate. The dye was precipitated with the silver chloride, leaving a clear yellow supernatant solution, and a clearly defined end-point was obtained. In the case of solutions containing neutral red, the salt was estimated by a method of steam distillation with sodium hydroxide, the concentration of salt in the experimental solution being calculated from estimations of the ammonia evolved.

The dyes were estimated colorimetrically, using a Hellige colorimeter, a brief description of which is given below. The solution to be analysed is carefully run into a small wedge-shaped glass trough which is supported alongside a wedge-shaped glass vessel fitted with a well-ground stopper and containing the standard solution. The two vessels are viewed through a Helmholtz double plate against a ground-glass background. The wedge containing the standard solution may be moved by means of a rack and pinion mechanism until the field, as seen through the double plate, presents a uniform tint. In such a position the colour intensity due to the thickness of the standard solution is equal to that of the solution in the trough. By using solutions of known concentration in the trough, a standard wedge was calibrated for each dye employed, and calibration curves were constructed. Preliminary experiments showed that, over the twenty-four hour period of an absorption experiment, the most suitable concentration of the dye in simple solution was 0·01 per cent. by weight, and the standard wedges were therefore filled with solutions of this concentration.

In early experiments, on attempting to estimate the percentage of dye in a solution in which mangold tissue had been immersed for approximately thirty-six hours, it was found impossible to match exactly the two halves of the field viewed through the double plate. On examination the solution appeared to be slightly turbid, and the slightest trace of turbidity materially affects the colorimetric estimation. This difficulty was not experienced in the case of solutions in which tissue had been immersed for twenty-eight hours, and a period of approximately twenty-four hours was adopted for each series of experiments.

In the usual method of procedure the jars containing the experimental solutions and sets of discs were suspended in the constant temperature bath at 20° C., and the only shaking they received occurred during the operation of taking a sample for analysis. Accordingly six jars were prepared containing the same experimental solution, a solution containing magnesium chloride and methylene blue, and three of the jars were placed in a shaker in a bath at 20° C., while the remaining three jars were suspended in the ordinary way. Analysis showed that the rate of intake of the dye was more rapid in the early stages of the process where the solution was kept in motion, but the ultimate result was the same in either instance. This effect of shaking is shown graphically in Fig. 1.

EXPERIMENTAL RESULTS.

As a preliminary to the investigation of the mutual influence of inorganic salts and dyes on their absorption by mangold tissue, it is necessary to obtain data with regard to the normal intake of the dyes and salts used when each of these is unaffected by the presence of the other in solution. The experimental results, therefore, fall into three groups, namely: (1) the absorption of the dyes by mangold tissue from pure dye solutions; (2) the absorption by the tissue from pure solutions of the inorganic salts; (3) the absorption of dye and salt by the tissue when immersed in solutions containing both dye and salt.

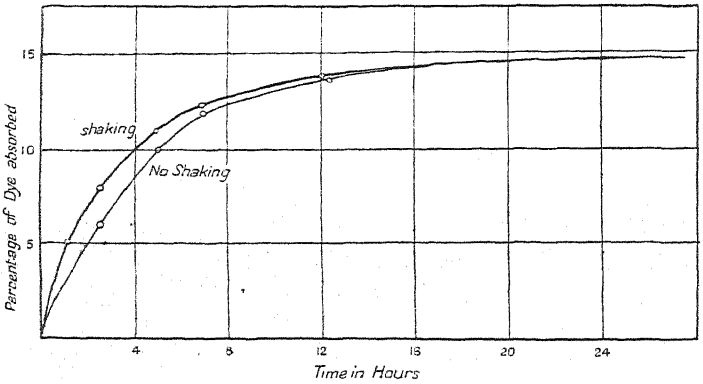

FIG. 1. The effect of constant shaking on the absorption of methylene blue by mangold from a solution containing magnesium chloride at 20° C.

1. *Absorption by Mangold Tissue from Pure Solutions of a Dye.*

The course of absorption by mangold was followed for three dyes, methylene blue, neutral red, and orange G; in the case of the first two dyes for a period of twenty-four hours, and in the case of orange G for approximately two days. These three dyes were chosen on account of their different properties. Methylene blue and neutral red are basic, while orange G is an acid dye. Methylene blue forms a true solution, while neutral red forms a semi-colloidal solution in water. The results are summarized in Table I. They show that the acid dye orange G is absorbed to a very much less extent than the basic dyes, while neutral red is absorbed to a considerably greater extent than methylene blue. The course of absorption is, on the whole, similar to that previously found for the absorption of a number of dyes by carrot (Redfern, 14).

TABLE I.

Intake of Methylene Blue, Neutral Red, and Orange G by Mangold Tissue, from 0·01 per cent. Solutions of the Dyes at 20° C.

Experimental Solution.	*Time in Hours.*	*Colorimeter Reading.*	*Percentage of Dye absorbed.*
Methylene blue, 0·01 %	0·683	23·13	19·2
	0·733	27·1	21·8
	1·43	41·0	31·4
	1·6	40·0	30·5
	3·93	57·8	45·4
	4·07	59·7	47·0
	7·12	66·1	53·6
	7·23	66·17	54·0
	24·0	76·0	64·5
	24·0	75·0	63·5
Neutral red, 0·01 %	1·35	30·85	17·0
	1·52	33·0	19·7
	5·0	51·6	42·8
	5·0	53·3	44·6
	12·08	76·0	74·0
	12·08	76·0	74·0
	24·75	86·0	85·0
	24·88	85·0	84·0
Orange G, 0·01 %	1·63	7·67	7·7
	8·2	8·03	8·0
	20·23	10·00	10·0
	50·0	12·7	12·7

The results tabulated above are graphically represented in Fig. 2.

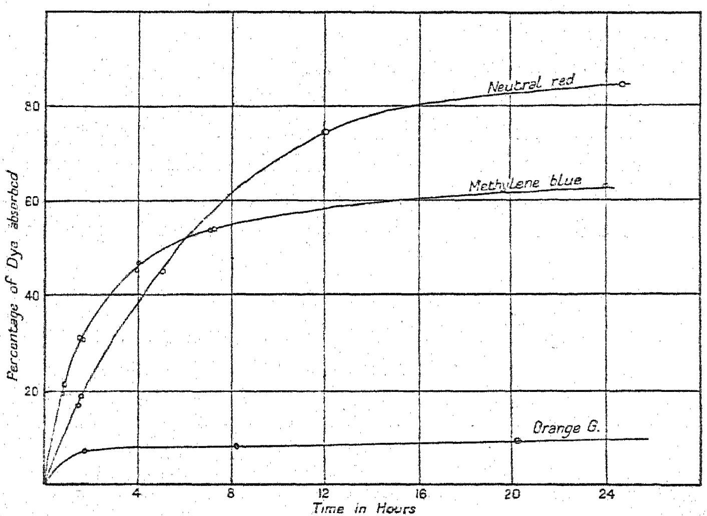

FIG. 2. The absorption of dyes by discs of mangold tissue immersed in 0·01 per cent. solution at 20° C.

It will be observed from Fig. 2 that the absorption of the dye approaches equilibrium in each case. The position of equilibrium is such that the concentration of the dye within the tissue is far greater than the concentration in the external solution. This 'heaping up' of the dye within the cell is quite characteristic and has been noted in former investigations of the intake of dyes by living tissues. Among previous observations of this phenomenon may be mentioned those of Szücs (19) and the later experiments of Miss Redfern (14).

2. *Absorption by Mangold Tissue from Pure Solutions of Inorganic Salts.*

The course of absorption by mangold tissue of the chlorides of ammonium, magnesium, and aluminium was followed in decinormal solutions of the three salts. Further determinations were made on the course of absorption in more dilute solution in the case of ammonium chloride. It should be stated at the outset that the results obtained by a method of chemical analysis of the external solution probably do not represent the true state of affairs. That the ions of a salt in solution may be absorbed at different rates and to different extents by living cells has been previously observed. Pantanelli and Sella (11) have shown that roots of living plants absorb the ions of salts, present in a culture solution, to different extents. Using isolated tissues, in a manner similar to that employed in the present experiments, Meurer (8) showed that discs of living carrot and beet tissue absorbed the ions of a salt in solution to different extents. Since in the experiments recorded in this section only the chloride present in the solution was estimated, the results presented in Table II relate only to the extent of absorption of the chloride ion. The results obtained indicate that absorption is practically the same from decinormal solutions of ammonium and aluminium chlorides, but is considerably slower in the case of magnesium chloride. That absorption is relatively more rapid from more dilute solutions is clearly shown in the case of ammonium chloride.

3. *Absorption by Mangold Tissue from Solutions containing a Dye and an Inorganic Salt.*

(i) Influence of ammonium chloride on the absorption of methylene blue.

Solutions containing 0·01 per cent. methylene blue and ammonium chloride in concentrations of 0·1 N., 0·075 N., 0·05 N., 0·025 N., and 0·01 N. were prepared and standardized with silver nitrate as previously described and also tested colorimetrically. The analyses of salt concentration in the solution at recorded intervals were performed on three samples of 10 c.c. each drawn from the jars. Experiments were conducted in duplicate or triplicate for each solution and 200 c.c. of solution with forty discs of mangold tissue were used in each jar. The results obtained are summarized in Table III and graphically represented in Fig. 4.

TABLE II.

The Absorption of Chlorides from Solution by Mangold Tissue at 20° C.

Solution.	*Time in Hours.*	*Initial Concentration in Normalities.*	*Estimated Concentration in Normalities.*	*Percentage of Salt absorbed.*
Ammonium chloride	—	0·1	—	—
	2·60		0·0986	1·40
	4·20		0·0981	1·90
	12·90		0·0974	2·55
	26·02		0·0972	2·8
”	—	0·05	—	—
	3·17		0·0486	2·80
	4·35		0·0484	3·20
	28·17		0·0475	5·0
Magnesium chloride	—	0·1	—	—
	1·07		0·0998	0·2
	3·93		0·0995	0·5
	4·85		0·0995	0·5
	19·57		0·0986	1·4
	20·7		0·0983	1·7
Aluminium chloride	—	0·09925	—	—
	1·03		0·0984	0·85
	4·22		0·0975	1·76
	12·03		0·0967	2·56
	28·0	(Tissue dead)	—	—

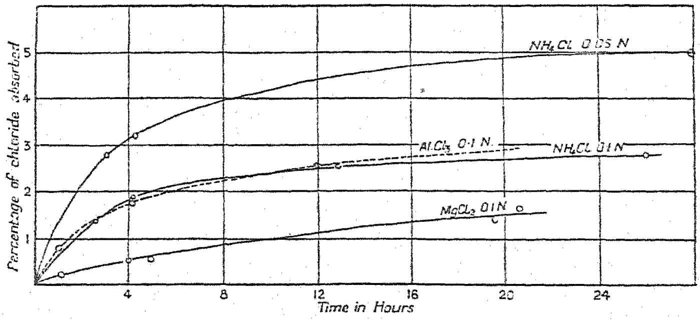

FIG. 3. The absorption of chloride by mangold from solutions of the salts at 20° C.

It becomes clear from the results represented in Fig. 4 that the concentration of the salt has a direct influence on the absorption of the dye, the extent of absorption of the dye decreasing with increasing concentration of the salt.

TABLE III.

The Absorption of Methylene Blue and Ammonium Chloride from Mixed Solutions by Mangold Tissue at 20° C.

Solution.	*Time in Hours.*	*Methylene Blue Estimation.*		*Ammonium Chloride Estimation.*		
		Reading on Colorimeter.	*Percentage of Dye absorbed.*	*Original Concentration.*	*Estimated Concentration.*	*Percentage of NH_4Cl absorbed.*
Ammonium chloride,	0·733	9·83	9·0	0·0985	0·0980	0·52
0·1 N.	6·53	25·7	21·0		0·0970	1·56
Methylene blue,	8·48	27·0	22·3		0·0965	2·13
0·01 %	25·07	32·0	25·0		0·0964	2·15
Ammonium chloride,	0·5	8·0	7·5	0·07425		
0·075 N.	2·0	18·0	15·5		0·07265	2·15
Methylene blue,	6·43	25·0	20·5		0·07227	2·66
0·01 %	25·43	34·0	26·1		0·072	3·03
Ammonium chloride,	1·33	15·5	13·5	0·04988	0·0489	2·0
0·05 N.	5·95	33·0	25·5		0·04807	3·62
Methylene blue,	28·95	52·5	40·5		0·0476	4·58
0·01 %						
Ammonium chloride,	0·7	17·0	14·8	0·02525	—	
0·025 N.	1·0	19·7	17·0		0·02472	2·1
Methylene blue,	1·68	30·0	23·5		0·02490	1·4
0·01 %	6·47	50·0	38·3			
	8·70	52·5	40·5		0·0243	3·8
	21·92	61·0	48·5		0·02425	4·0
Ammonium chloride,	1·0	28·0	22·2			
0·01 N.	3·0	33·7	26·2			
Methylene blue,	6·82	55·0	42·6			
0·01 %	8·50	62·0	49·5			
	21·89	71·0	59·0			

(ii) The influence of ammonium chloride on the absorption of neutral red.

In Table IV are summarized the results of a series of experiments in which the dye used was neutral red. Ammonium chloride solutions of the same concentrations as those employed in the case of methylene blue were used. In estimating the percentage of salt absorbed it was necessary to remove a larger volume of the solution than was removed in the previous series with methylene blue. The error introduced in this way was therefore considerably greater, and only in the first instance, in the solution containing approximately 0·1 N. ammonium chloride, were analyses made at intervals. In the remaining solutions the percentage of ammonium chloride absorbed was estimated at the conclusion of each experiment.

It should be observed that the method of estimation of ammonium chloride in the presence of neutral red involves its estimation as ammonia. As the ammonium ion and the chloride ion need not necessarily be absorbed to the same extent (11), the results so obtained are not directly comparable with those obtained by analyses of the solution using silver nitrate. It appears, however, that in the case of ammonium chloride the NH_4 ion is more rapidly absorbed than the Cl ion by mangold tissue. The results tabulated above and graphically represented in Fig. 5 show that the absorption of neutral red by the discs of mangold is not retarded by the presence of ammonium chloride to the same extent that the intake of methylene blue is retarded by this salt.

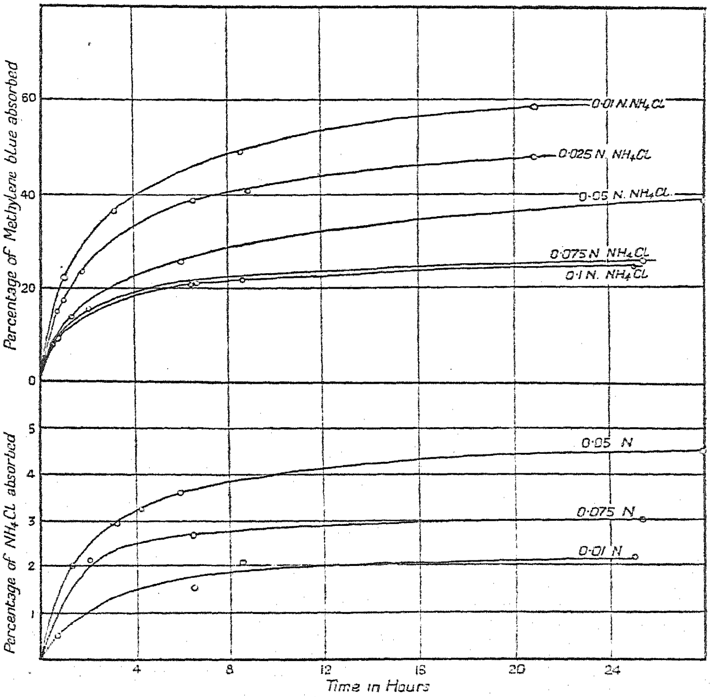

FIG. 4. The absorption of methylene blue and ammonium chloride from solutions containing 0·01 per cent. of the dye and various concentrations of the salt.

(iii) Influence of ammonium chloride on the absorption of orange G.

A solution containing orange G and ammonium chloride was prepared in which the respective concentrations of the two substances were 0·01 per cent. and 0·1 N. Since after three days' immersion in a single solution of the dye in the same concentration forty discs of mangold absorbed only 12 per cent. of the dye, it is to be expected that, in a solution containing a salt, only slight absorption will occur. That such is indeed the case was revealed in the course of experiments in which the above solution was employed. After a period of twenty-four hours no measurable absorption of dye had occurred. Even after three days, though the discs of tissue remained quite healthy and turgid, absorption of the dye did not exceed 3 per cent.

TABLE IV.

The Absorption of Neutral Red and Ammonium Chloride from Mixed Solutions by Mangold Tissue at 20° C.

	Estimation of Neutral red.			*Estimation of NH_4Cl.*			
Solution.	*Time in Hours.*	*Colorimeter Reading.*	*Percentage absorbed.*	*Time in Hours.*	*Original concentration in Normalities.*	*Estimated concentration in Normalities.*	*Percentage absorbed.*
Ammonium chloride,	4·67	46·7	36·2	1·5	0·0981	0·0974	0·61
0·1 N.	6·27	52·1	43·3	5·22		0·09575	2·3
Neutral red,	11·48	61·0	54·5	24·0		0·09575	2·3
0·01 %	24·58	64·5	59·5				
Ammonium chloride,	1·05	34·0	21·0		0·075		
0·075 N.	6·6	58 0	50·3				
Neutral red,	13·8	70·3	67·0	13·8		0·0730	2·67
0·01 %	27·03	78·0	76·0	27·03		0·0722	3·75
Ammonium chloride,	2·84	52·4	43·5		0·05		
0·05 N.	4·75	60·56	54·0				
Neutral red,	12·57	71·2	68·3				
0·01 %	24·68	82·0	80·0	24·68		0·0466	6·8
Ammonium chloride,	0·75	36·2	23·4		0·0098		
0·01 N.	2·53	58·43	43·6				
Neutral red,	4·9	61·0	54·5				
0·01 %	6·2	72·0	68·2				
	12·27	63·0	81·6				
	13·32	84·0	83·0				
	28·32	90·0	90·0	28·32		0·0088	9·9

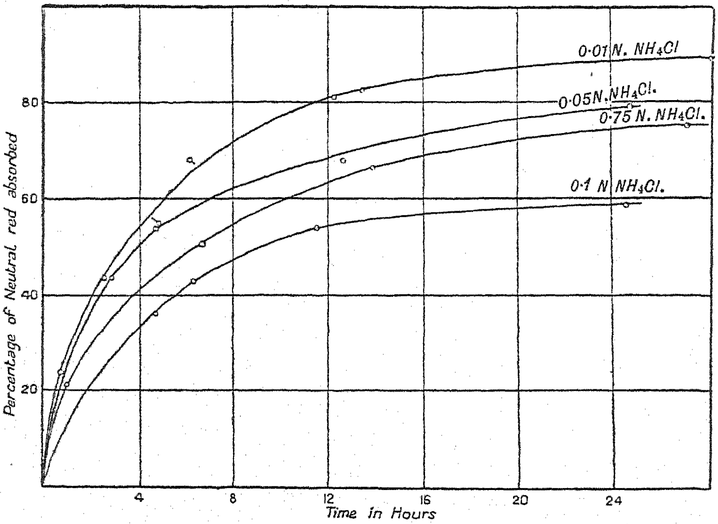

FIG. 5. The absorption of neutral red from solutions containing ammonium chloride in various concentrations by mangold at 20° C.

(iv) Influence of magnesium chloride on the absorption of methylene blue.

Similar experiments to those above described were performed using solutions containing magnesium chloride the absorption of dye alone being estimated.

Table V.

The Absorption of Methylene Blue from Solutions containing Magnesium Chloride by Mangold Tissue at 20° C.

Solution.	*Time in Hours.*	*Colorimeter Reading.*	*Percentage of Dye absorbed.*
Magnesium chloride, 0·1 N. Methylene blue, 0·01 %	3·0	3·0	3·0
	4·97	5·0	5·0
	12·82	5·2	5·15
	24·9	7·0	6·6
Magnesium chloride, 0·05 N. Methylene blue, 0·01 %	1·17	slight absorption	
	2·58	6·0	6·0
	6·88	12·0	10·7
	24·47	13·0	11·5
Magnesium chloride, 0·01 N. Methylene blue, 0·01 %	1·05	15·3	13·3
	4·05	25·0	20·5
	9·73	35·0	27·0
	13·47	40·0	30·5
	25·12	43·0	33·0

(v) Influence of magnesium chloride on the absorption of neutral red.

Table VI gives the results obtained in three series of experiments where the experimental solutions of 0·01 per cent. neutral red contained magnesium chloride in concentrations of 0·1 N., 0·05 N., and 0·01 N. respectively.

Table VI.

The Absorption of Neutral Red by Mangold Tissue from Solutions containing Magnesium Chloride at 20° C.

Solution.	*Time in Hours.*	*Colorimeter Reading.*	*Percentage of Dye absorbed.*
Magnesium chloride, 0·1 N. Neutral red, 0·01 %	1·2	22·0	10·0
	5·42	43·5	32·0
	6·7	50·2	41·0
	12·0	54·23	46·0
	24·0	56·8	49·0
Magnesium chloride, 0·05 N. Neutral red, 0·01 %	0·92	33·9	21·0
	6·28	53·0	44·3
	18·37	61·43	55·2
	24·3	67·3	63·3
Magnesium chloride, 0·01 N. Neutral red, 0·01 %	0·5	23·1	10·3
	5·82	65·6	61·0
	18·05	77·7	75·8
	24·0	82·05	80·5

(vi) and (vii) The influence of aluminium chloride on the absorption of methylene blue and neutral red.

Similar series of experiments were performed using aluminium chloride in the dye solutions. When present in concentration of 0·05 N. this salt inhibits the intake of methylene blue almost completely, only the outer surface of the discs of mangold tissue being at all coloured. Sections of a disc were examined microscopically and showed that the penetration of the dye was confined to the periphery of the tissue only. This strongly antagonistic property of aluminium has been demonstrated by numerous investigators previously, and the results obtained, presented in Tables VII and VIII, are fully in accord with previous observations.

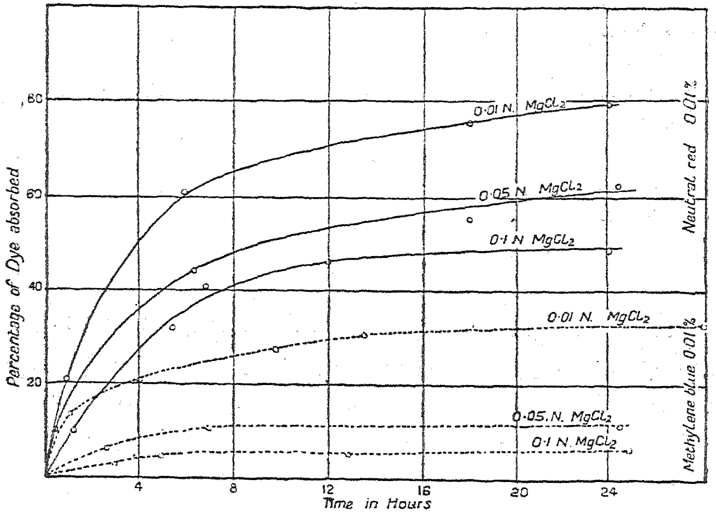

FIG. 6. The absorption of methylene blue and neutral red by mangold from solutions containing magnesium chloride in various concentrations at 20° C.

Curves were constructed from the results summarized in the above tables, but, since they exhibited essentially the same form as those given for the absorption of the dyes in the presence of magnesium chloride, they have not been included here. On comparing the figures obtained for the percentage of neutral red absorbed in the presence of magnesium and aluminium chlorides respectively, it would appear that the antagonistic action of aluminium chloride when present in a concentration of 0·001 N. is approximately equal to that of magnesium chloride at fifty times this concentration.

It is, however, well known that aluminium chloride may be hydrolysed in aqueous solution according to the equation $AlCl_3 + 3H_2O \rightarrow Al(OH)_3 + 3HCl$. This being so, in a solution standardized in the usual way using silver nitrate there may be present an appreciable quantity of free hydrochloric acid, while a corresponding quantity of aluminium hydroxide is present. The consequence of this property is that in a given solution of the salt the actual number of aluminium ions available is less than the concentration indicates, as aluminium hydroxide is colloidal. An attempt was made to discover whether by employing the chloride of a trivalent metal such as lanthanum, which does not undergo hydrolysis, any difference in antagonizing power was revealed.

TABLE VII.

The Absorption of Methylene Blue by Mangold Tissue from Solutions containing Aluminium Chloride in various Concentrations at 20° C.

Solution.	*Time in Hours.*	*Colorimeter Reading.*	*Percentage of Dye absorbed.*
Aluminium chloride, 0·1 N.	1·6	—	Trace
Methylene blue, 0·01 %	4·15	—	,,
	12·05	—	,,
	24·08	—	,,
Aluminium chloride, 0·05 N.	1·53	—	Trace
Methylene blue, 0·01 %	4·1	—	,,
	12·0	—	,,
	24·0	2·5	2·5
Aluminium chloride, 0·01 N.	1·85	—	Trace
Methylene blue, 0·01 %	3·50	3·0	3·0
	11·22	5·0	5·0
	23·37	10·3	9·3
Aluminium chloride, 0·005 N.	2·42	4·0	4·0
Methylene blue, 0·01 %	6·0	11·46	10·4
	10·2	14·4	12·6
	24·4	29·04	23·0
Aluminium chloride, 0·001 N.	1·12	21·17	18·0
Methylene blue, 0·01 %	2·22	27·17	22·0
	3·05	33·8	26·0
	4·67	41·1	31·4
	7·83	48·2	37·0
	21·50	68·0	55·7
	24·18	70·8	59·0

(viii) and (ix) Influence of lanthanum chloride on the absorption of methylene blue and neutral red.

Solutions of lanthanum chloride and the dyes were prepared and four series of experiments performed, two with methylene blue and two with neutral red, the results of which are presented in Tables IX and X.

On comparing the results obtained with lanthanum chloride with those obtained with aluminium chloride used in the same concentrations it appears that the former has a stronger antagonizing effect than the latter in solutions containing methylene blue. In the case of neutral red this difference is not shown, the antagonistic properties of the two salts towards this dye being slightly greater in the case of aluminium chloride. As with magnesium chloride, curves were constructed from the data presented in Tables IX and X and the same general form was exhibited.

TABLE VIII.

The Absorption of Neutral Red by Mangold Tissue from Solutions of the Dye containing various Concentrations of Aluminium Chloride at 20° C.

Solution.	*Time in Hours.*	*Colorimeter Reading.*	*Percentage of Dye absorbed.*
Aluminium chloride, 0·05 N.	0·88	5·0	1·5
Neutral red, 0·01 %	1·17	8·0	2·6
	4·70	10·1	3·45
	13·0	14·3	5·6
	28·1	14·6	6·0
Aluminium chloride, 0·01 N.	3·0	22·0	10·0
Neutral red, 0·01 %	6·8	24·7	11·8
	9·48	26·0	12·8
	24·42	31·25	18·0
Aluminium chloride, 0·005 N.	2·4	20·7	9·15
Neutral red, 0·01 %	4·23	23·93	11·0
	12·5	30·8	17·5
	27·55	37·2	25·5
Aluminium chloride, 0·001 N.	3·0	45·0	34·0
Neutral red, 0·01 %	6·57	59·0	52·0
	9·22	63·0	57·3
	24·25	74·86	72·2

TABLE IX.

The Absorption of Methylene Blue by Mangold Tissue from Solutions containing Lanthanum Chloride at 20° C.

Solution.	*Time in Hours.*	*Colorimeter Reading.*	*Percentage of Dye absorbed.*
Lanthanum chloride, 0·01 N.	3·5	Trace	Trace
Methylene blue, 0·01 %	6·2	3·0	3·0
	11·2	4·5	4·5
	24·32	8·9	8·3
Lanthanum chloride, 0·001 N.	1·37	16·1	14·0
Methylene blue, 0·01 %	3·20	28·1	22·5
	5·80	34·9	26·95
	19·10	51·0	39·0
	23·1	56·5	44·0

From the curves constructed from the experimental data presented in the foregoing tables the approximate percentage of dye absorbed after a period of twelve hours has been obtained in each case, and the results are collected in Table XI.

Table X.

The Absorption of Neutral Red by Mangold Tissue from Solutions containing Lanthanum Chloride at 20° C.

Solution.	*Time in Hours.*	*Colorimeter Reading.*	*Percentage of Dye absorbed.*
Lanthanum chloride, 0·01 N. Neutral red, 0·01 %	1·35	11·1	4·0
	4·82	23·93	11·0
	6·58	27·8	14·5
	11·85	33·17	19·6
	24·85	43·2	31·4
Lanthanum chloride, 0·001 N. Neutral red, 0·01 %	3·68	46·0	35·3
	6·37	56·4	49·0
	11·72	67·0	63·0
	24·86	73·5	71·0

Table XI.

The Absorption of Methylene Blue and Neutral Red by Mangold Tissue from 0·01 per cent. Solutions of the Dyes containing the Chlorides of Ammonium, Magnesium, Aluminium, and Lanthanum in various Concentrations at 20° C.

Salt Present.	*Concentration in Normalities.*	*Time in Hours.*	*Percentage of Dye absorbed.*	
			Methylene blue.	*Neutral red.*
Ammonium chloride	0·1	12	23·8	56·0
	0·075	12	23·8	66·0
	0·05	12	29·5	68·0
	0·025	12	43·5	72·0
	0·01	12	55·0	81·0
Magnesium chloride	0·1	12	6·9	46·0
	0·05	12	11·0	52·0
	0·01	12	29·0	70·5
Aluminium chloride	0·1	12	—	2·0
	0·05	12	1·5	5·0
	0·01	12	5·0	14·0
	0·005	12	15·0	18·0
	0·001	12	42·0	61·0
Lanthanum chloride	0·01	12	5·5	20·0
	0·001	12	39·0	64·0

The results tabulated above are graphically represented in Figs. 7 and 8.

DISCUSSION OF EXPERIMENTAL RESULTS.

1. *The Course of Absorption.*

In general, the curves obtained for the absorption of the dyes used in this investigation agree with those obtained by Miss Redfern (14), who estimated the intake of dyes by discs of carrot tissue, by a method essentially similar to that employed with mangold. The same investigator noted that acid dyes were not absorbed by carrot to any appreciable extent. Results obtained in experiments with orange G entirely support this observation, and the small tendency exhibited by living storage tissues to absorb acid dyes is again illustrated. This property of acid dyes was mentioned by Pfeffer (12) in his early investigations of the absorption of dyes by living cells, and later formed the subject of an investigation by Collander (1).

A comparison of the curves obtained for the absorption of methylene blue and neutral red indicates a difference in the nature of the absorption of these two dyes. In the early stages the rate of absorption of methylene blue is more rapid than that of neutral red, but the rate diminishes more rapidly with time, till eventually, after a period of twenty-four hours, the quantity of neutral red absorbed is greater than that of methylene blue.

Sufficient attention has not always been drawn in the past to the difference between the rate of entrance, or, more correctly, the rate of equilibration, and the position of equilibrium.

Some explanation of these differences may be sought in the specific properties of the dyes which might be expected to affect the intake of the dye when presented to living tissue in simple solution or in mixed solutions with other substances. Classified according to the size of the molecule, neutral red may be termed a semi-colloid, while methylene blue is a crystalloid. This difference in size of the molecules may in itself have a direct bearing upon the rate at which either enters a living cell. An observation made by Pfeffer (12) is also of interest in connexion with the absorption of methylene blue. It was found that methylene blue, when taken up by a living cell, was retained almost entirely within the vacuole, the protoplasm of the cell remaining unstained. This was not found to occur with any other dye examined; in all other cases where the dye entered the cell, both the protoplasm and the vacuole contained the dye. It has been urged by certain investigators, among whom may be mentioned Moore and Roaf (9), that the essential process in the absorption of substances by living cells, is a process of adsorption. Were this so, it would be reasonable to expect that a substance such as methylene blue would be found in greater concentration in the highly colloidal protoplasm rather than in the watery contents of the vacuole. That adsorption of the dye is a possibility in the case of neutral red receives some support from experiments by Szücs (19) on the intake of dyes by *Lemna minor*. It was found that neutral red was absorbed to a greater extent than other dyes used. Ruhland (15) attributed this apparent greater intake of neutral red to the fact that adsorption of the dye occurred in the cell-wall, and when this was prevented by the presence of hydroxyl ions in the solution, the intake of neutral red was found to conform with other cases investigated.

2. *The Antagonistic Action between Inorganic Salts and Dyes.*

When a dye is presented to living tissues in solutions containing ammonium chloride, the results presented in Table III show clearly that the rate and ultimate extent of absorption is reduced. That this is a phenomenon of general occurrence is confirmed by the results obtained with magnesium, aluminium, and lanthanum chlorides, and this antagonistic action between an electrolyte and a non-electrolyte seems to be of the same nature as the antagonism observed between the ions of inorganic salts. The results obtained are in agreement with those of Szücs (20), who suggested that the reduction in the absorption of one substance by the addition of a second substance to the solution might be explained on grounds of mutual hindrance. Thus if a particular substance or surface is available for the absorption of a dye, and the same substance or surface is also essential for the absorption of the ion of a salt, it is reasonable to suppose that neither dye nor salt will be absorbed to the fullest possible extent in the presence of the other. At the same time, if this mutual hindrance merely takes place in a limiting cell membrane, it also appears reasonable to suppose that it will affect only the rate of equilibration, and not the actual position of equilibrium. The results obtained with ammonium chloride and methylene blue support the view that antagonism is due to a mutual hindrance to absorption, but the curves obtained from these results show that absorption of dye and salt approaches a position of equilibrium lower in both cases than that which would be attained by either in the absence of the other (see Figs. 2, 3, and 4).

The antagonistic action occurring between salt and dye is less emphasized between neutral red and an electrolyte than between methylene blue and the same salt. This difference is quite in agreement with the difference noted in the absorption of the two dyes from simple solution, and may in the same way be attributed to the specific properties of the dyes in question.

3. *The Influence of Concentration of Salt in reducing the Intake of Dye.*

That reduction in the absorption of the dye by a salt in the solution is influenced by the concentration of the salt present is shown in each instance. The results obtained for the absorption of a dye after twelve hours when presented to mangold tissue in solutions containing salts in various concentrations are given in Figs. 7 and 8. These figures show clearly the relationship between the concentration of any one of the chlorides employed and the absorption of the dye from mixed solutions. A comparison of the results obtained with methylene blue and neutral red reveals again that the antagonistic effect of equal concentrations of ammonium chloride is greater in the case of methylene blue. Indeed, in low concentrations of electrolyte there appears to be a slight increase in absorption of the dye above the rate of absorption from a simple solution. The possibility of slight variability in the tissues used in different experiments renders it inadvisable to lay too much stress on this point, but the work of Endler (4) may be mentioned in this connexion. Investigating the intake of methylene blue and neutral red by various algae, Endler states that the amount of dye absorbed increased at first with concentration of the electrolyte, but further increase in the concentration produced the reverse effect.

That the presence of the dye has a slight retarding effect on the intake of the salt is seen on comparing the estimations of ammonium chloride absorbed by mangold tissue in the presence of a dye with the estimations of the intake of the salt from pure solutions. The antagonistic action of the dye towards the salt is very slight, a result which would be expected when the relative molecular concentrations of dye and salt are compared.

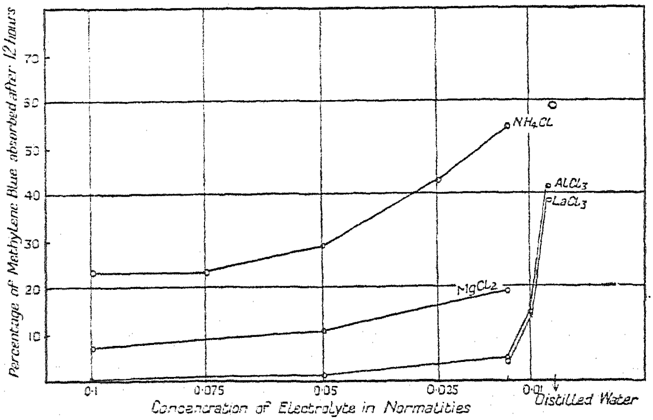

FIG. 7. The influence of the presence of the chlorides of ammonium, magnesium, aluminium, and lanthanum in various concentrations on the absorption of methylene blue by mangold at 20° C.

The results obtained with magnesium chloride in various concentrations are essentially similar to those obtained with ammonium chloride. The absorption of methylene blue and neutral red is antagonized in the presence of magnesium chloride, the degree of antagonism bearing a definite relationship to the concentration of the salt and being greater towards methylene blue. The results obtained with the chlorides of aluminium and lanthanum are in agreement with those obtained with ammonium and magnesium chlorides. In each series the antagonism exhibited by the electrolyte towards methylene blue was greater than that towards neutral red.

The influence of salt concentration on the absorption of the dye suggests that the same substance is involved in the absorption of both dye and salt.

4. *The Influence of the Valency of the Kation in reducing the Intake of Dye.*

Figs. 7 and 8, which are in the nature of a summary of results obtained in this investigation, clearly show that the antagonizing power of any one of the chlorides employed is a function of the valency of the kation and the concentration, a conclusion wholly in accord with those obtained by different methods of experiment. This influence of the valency of the kation was previously noted by Szücs (19, 20), and held to support the adsorption theory. Support on this ground is only forthcoming if it is established that the higher the valency of the salt, the greater is the extent to which it is absorbed. The experimental results obtained indicate that this is not so, for aluminium and ammonium chlorides are absorbed to approximately equal extents, while magnesium chloride is absorbed to a considerably less extent than either.

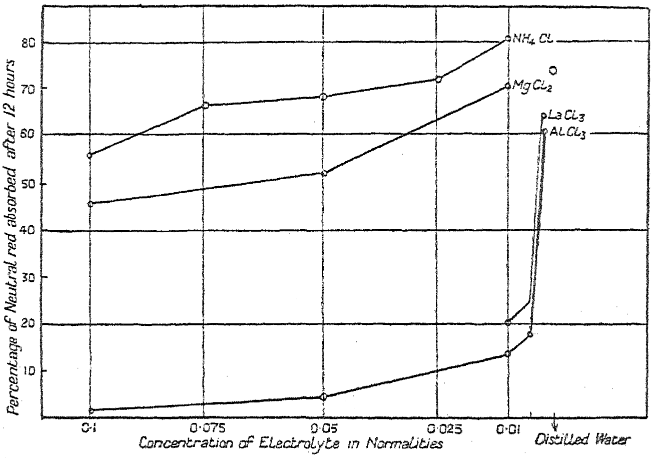

FIG. 8. The influence of the presence of the chlorides of ammonium, magnesium, aluminium, and lanthanum in various concentrations on the absorption of neutral red by mangold at 20° C.

The antagonistic action of lanthanum as compared with that of aluminium is worthy of comment. As previously indicated, the hydrolysis of aluminium in aqueous solution and the consequent liberation of the monovalent anions in place of the trivalent aluminium ion (present in colloidal aluminium hydroxide) might be expected to reduce the antagonistic properties of a solution of this salt. Consequently an equivalent solution of the chloride of a trivalent metal which does not undergo hydrolysis in this way would probably have a slightly higher antagonistic value. That such is indeed the case is shown by the effect of lanthanum chloride on the intake of methylene blue by mangold, although the reverse effect is shown in the case of neutral red.

5. *The Bearing of the Results obtained in Relation to the Mechanism of Cell Permeability.*

In early work on the antagonistic action of calcium to the toxic effect of sodium chloride towards the developing eggs of *Fundulus*, Loeb (6) found that the antagonistic effects occurred only when the fish was surrounded by a membrane. He therefore concluded that the membrane was the seat of antagonistic action, and that the ions of calcium and sodium mutually hindered each other in the passage through the membrane. It is usually supposed that in plants the seat of antagonism is also a membrane, though one of a different nature, namely, the plasma membrane, which forms the limiting layer of the living protoplast. The present view of the nature of this limiting layer of the protoplasm is that it is determined by the composition of the bulk of the protoplasm and the external medium, for all substances tending to lower the surface tension will accumulate at the surface. Alteration in the composition of this limiting layer may therefore be effected by changes within the cell or changes in the external medium. At the same time alterations in the composition of the limiting layer, it may be supposed, are likely to bring about a change in the permeability of the layer towards substances on either side of it. Ruhland (15) has suggested that this limiting layer of the protoplasm acts as a molecular sieve, the entrance or non-entrance of a substance being determined by the size of its molecular aggregates. Such a view receives support from the experiments conducted with methylene blue and neutral red in simple solution. The curves obtained indicate that neutral red enters the cell at a considerably slower rate. Since fatty substances tend to lower surface tension, on the view of the nature of the plasma membrane formulated above, it has been suggested that the limiting layer of the protoplasm is composed of fatty or lipoid substances. Of the dyes employed, only neutral red was found to enter a lipoid substance, castor oil, from a solution in water. If the dyes passed through a lipoid phase in the membrane, as neutral red is soluble in such a phase it would in all probability enter the cell more rapidly than methylene blue. That this is not the case is seen from the curves in Fig. 2. On the other hand, the position of equilibrium suggests that adsorptive or chemical processes are operative in the course of absorption of the dye. A view propounded by Kahho (5) as to the nature of penetration of the cell by substances present in the external medium is of interest in connexion with the relationship existing between the degree of antagonism and the valency of the antagonizing ion. According to this view, the power of penetration of a substance is related inversely to its capacity for coagulating certain of the cell colloids. Coagulation of these colloids renders the outer layers of the protoplasm less permeable to substances in external solution. In the precipitation of colloids by electrolytes, the ion bringing about coagulation is the one carrying a charge of opposite sign to that on the colloidal particles. Efficiency in coagulation by an electrolyte of a colloid of negative sign depends on the valency of the kation of the salt, and is often independent of the nature of this kation. That the kations of the salts employed may have caused some such coagulation of the protoplasm in the manner suggested seems distinctly possible, and might be used to explain the relationship existing between the valency of the kation and the antagonizing action it exerts.

It has recently been shown by Collander (2), in an investigation of the permeability of copper ferrocyanide precipitation membranes to non-electrolytes, that although the permeability of such membranes is mainly due to their acting as molecular sieves, yet solution in the membrane may also occur, and so mask considerably the sieve action of the membrane. There are clearly similar possibilities in the case of the limiting layer of the protoplasm, and a possible explanation of antagonism may rest on such a basis.

In general, the conclusions reached in the present investigation appear to support, in part at least, three views of the mechanism of cell permeability. In the first place, the course of absorption of the dyes, taking into consideration the size of the molecule, affords support for the ultra-filtration theory. It has already been pointed out that the phenomena of antagonism afford a large measure of support to the colloid precipitation theory. Kahho (5) suggests that the particular colloidal particles precipitated are probably the lipoid constituents of the limiting layer of the protoplasm. In order that these colloidal particles may be precipitated by kations of electrolytes it would be necessary for them to carry a negative charge. That these particles do carry a negative charge in the cells of root storage tissue is supported by the fact that basic dyes are in general readily absorbed. Since the colour group of a basic dye carries a positive charge, the existence of negatively charged particles in the limiting layer of the protoplasm, would tend to forward absorption of the dye. Thirdly, the results appear to agree well with Raber's theory (13) according to which permeability is due to the electrical condition of the semi-permeable membrane, which in the tissue used by Raber, as in that employed in the work described above, is supposed to carry a negative charge. The mechanism of dye absorption suggested by Raber is, however, quite different from that suggested above.

SUMMARY.

1. The absorption of methylene blue, neutral red, and orange G respectively has been followed in simple solution and in solutions containing inorganic salts, namely, the chlorides of ammonium, magnesium, aluminium, and lanthanum.

2. In simple solution the absorption of the dye can be explained on the sieve theory (ultra-filtration theory) of the nature of the plasma-membrane.

3. The equilibrium is such that a 'heaping up' of the dye in the cell is observed, and the continued absorption of the dye when the internal concentration of dye must be very much higher than the external concentration may be explained on the ground of adsorption or chemical combination, which may occur with some substance in the cell.

4. The presence of a salt in a solution of a dye antagonizes the intake of the dye by living tissue. The magnitude of the antagonistic action is a function of the valency of the salt and its concentration. This conclusion is similar to that arrived at by Szücs.

5. The antagonistic action between an electrolyte and methylene blue was, in all the cases investigated, stronger than that between the same electrolyte and neutral red.

6. The colloid precipitation theory of the plasma-membrane as formulated by Kahho receives support from phenomena of antagonism investigated, and affords a possible explanation of the relationship existing between the degree of antagonism and the valency of the kation of the antagonizing salt.

7. The results obtained in this investigation are not inconsistent with Raber's view that the plasma-membrane carries an electric charge.

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READING.

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Water Content, a Factor in Photosynthesis.

BY

R. H. DASTUR.

With one Figure in the Text.

INTRODUCTION.

LEAVES are important organs of plants and are almost entirely responsible for the production of carbohydrates which form an essential part of the food of plants. The carbohydrates manufactured by the leaves from the carbon dioxide of the air and the water from the soil, by processes which are still quite obscure, and the complex substances produced therefrom, are utilized by plants for the purposes of growth and the production of flowers, fruits, and seeds. A period is, however, reached when the functional activities of a leaf cease and it is no longer able to synthesize sugar or starch, and it slowly begins to lose its turgidity and to droop, being unable to support its own weight, and is finally severed from the plant either by the pressure of the wind or by the formation of absciss layers at its base. The causes which thus deprive leaves of their function and terminate their existence are, at present, not properly understood. Light, optimum temperature, and an adequate water-supply are indispensable factors for the maintenance of their activities, but after a certain stage they no longer remain functional even under optimum conditions. They exhibit senescence, but a perennial plant is in no way worse for it, as new leaves continue to be formed at the growing-points, while the older leaves die and fall away. The capacity of continued growth and of regenerating the lateral members is a distinct advantage plants possess over members of the animal kingdom, by means of which they prolong their individual existence till they die or decay on account of natural causes.

The period during which leaves remain functional varies with different plants. The leaves of annuals remain active for the greater part of their existence, but the leaves of deciduous and evergreen trees have a limited duration and leaf fall occurs periodically. Some trees drop their leaves several times during a year, while some do so in a particular season only, remain leafless up to the end of that season, and produce fresh leaves in the

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next season. Though the time of abscission is dependent upon external factors and can be hastened by feeble illumination, an insufficiency of water, and sudden climatic changes, or can be delayed by favourable conditions, still under normal conditions leaves drop off in a regular manner at a definite period which varies with different leaves.

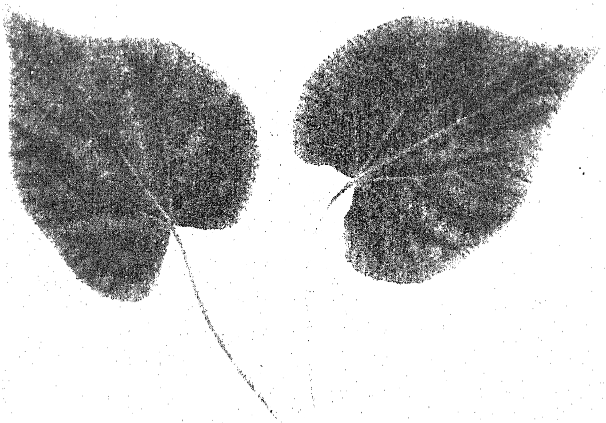
The chlorophyll content, water content, and some protoplasmic factor are three possible internal factors of photosynthesis, and failure of any one of them might lead to inhibition of the photosynthetic activity of the leaves and so finally to their decay. Willstätter and Stoll (13) are of opinion that the assimilatory process depends upon the chlorophyll content and some enzyme, and the increase in the chlorophyll content under certain conditions causes increase in assimilation. But the results obtained by them in experimenting with leaves are very complex and rule out the chlorophyll content as the internal factor controlling the photosynthetic activity.

Though not much work has been done to investigate the relation between the water-supply and photosynthetic activity, it has been shown by Kreusler (7) and Nagamatz (8) that the withdrawal of water from the leaf decreases its photosynthetic activity. Similar but more exact observations have been made by Thoday (11) with an improved half-leaf method, and the closure of stomata is ascribed by him as the cause of the lowered rate of photosynthesis when the water is withdrawn. Sachs (10) also favours the same view, which is supported by the fact that in plants without stomata, such as algae, lichens, and mosses, photosynthesis is much less depressed by loss of water than is the case in foliage leaves, as was shown by Klebs (6), Bastit (1), and Jumelle (5). But on the other hand, according to Treboux (12) and Pantanelli (9), the closure of the stomata cannot be the cause of the decline in photosynthesis with decreasing turgidity of the cells in the case of water plants. Iljin (4) has also made further observations similar to those made by Thoday (11), but his results do not show any proportionality between the photosynthetic velocity and water content.

It is conceivable, from the observations of the above-mentioned authors, that withdrawal of water can diminish the rate of photosynthesis, as water is necessary for photosynthesis and for keeping the photosynthetic elements in a normal condition for the discharge of their function. But it cannot be inferred from those observations that under natural conditions inadequate water-supply terminates the photosynthetic activity of a leaf. That such a relation between the water-supply and photosynthesis does exist in nature appears from the investigation here described.

INVESTIGATION.

It was discovered that in the mature leaves of *Abutilon asiaticum*, G. Don, starch was not formed uniformly all over the surface of the leaves, as was seen by macrochemical reaction with iodine, but was gradually disappearing from the marginal and apical portions of the leaves, and as the leaves grew older the photosynthetic decay appeared in the meshes of the vascular network, commencing from the margins towards the central portions (see figure below). At a later stage starch formation was restricted to the cells bordering the vascular network, and such a leaf when treated with iodine showed black streaks of starch lining the vascular network. Finally starch began to disappear from the finest veins to larger veins and then starch formation ceased entirely, thus terminating the activity of the leaves, which, however, remained attached to the plant for a long time. These changes, leading to the gradual decrease in the photosynthetic activity of the leaves, could be made out without difficulty on external examination. Distinct areas on the margin, apices, and in the meshes of the reticulate venations could be easily seen by holding the leaves against the light, and were distinguished readily on account of the yellow-green colour as contrasted with the dark green leaf surface. They gradually lost their greenish tint and turned yellow. The yellowing of the leaves proceeded exactly in the manner described and then spread all over the surface. Microscopical examination showed that the chloroplasts of the cells occupying these areas where starch formation had ceased had disintegrated, being broken up into small granules, and had turned yellow on account of the decomposition of the chlorophyll. It was clear from the above that the disintegration of chloroplasts preceded the disappearance of starch, and it first commenced in the cells which were placed farthest from the vascular tracheides and gradually extended to the neighbouring cells.



A photograph of the leaves of *Abutilon asiaticum*, G. Don, showing the marginal and intravascular decay. The leaves were extracted with alcohol and treated with iodine. The unstained regions indicate the cessation of functional activity.

This mode of photosynthetic decay was not peculiar to the leaves of *Abutilon asiaticum*, G. Don, and the same phenomenon has been observed in the leaves of many other plants. It was noticed in the leaves of *Ricinus communis*, Linn., *Carica Papaya*, Linn., *Malachra capitata*, Linn., *Corchorus olitorius*, Linn., *Hibiscus esculentus*, Linn., *Ipomoea hederacea*, Jacq., *Sparmannia africana*, Linn., a species of *Aristolochia*, and a species of *Abutilon*, and the series could be further extended. The leaves of some of these plants, such as *Carica Papaya*, Linn., and *Corchorus olitorius*, Linn., showed only marginal decay, and some showed both marginal and intravascular decay.

The characteristic manner in which the disappearance of starch was noticed suggested the possibility of a decline of water-supply due to the loss of conductivity in the smallest veins, and in order to test this view *Aristolochia* leaves of different ages were put with the cut ends of their petioles immersed in aqueous solutions of various dyes to see how far the dyes penetrated the vascular network. Aqueous solutions of different dyes were tried, such as 0·01 per cent. solution of fuchsin, 0·001 per cent. solution of safranin, 0·001 per cent. solution of methyl blue, and 0·005 per cent. solution of eosin. From among these the solution of eosin yielded good results, but the other dyes were not absorbed by the walls of the conducting elements and the veins were not stained. In young leaves of *Aristolochia*, where starch formation was normal, the veins were uniformly stained all over the leaf surface, but in older leaves the larger veins were more conspicuously stained, while the smaller veins occupying the marginal and central portions between the main veins were very faintly stained. The same leaves were tested for starch. In young leaves the starch was formed all over the surface, but in older leaves starch had disappeared from exactly those intravascular regions where the veins were feebly stained.

At the same time the leaves of *Sparmannia africana*, Linn., and a species of *Abutilon* were similarly tried and yielded the same results.

With a view to finding out whether the smaller veins underwent any change in structure as the leaves grew older, the smallest tracheides from the corresponding regions of the leaves of different ages were dissected out and microscopically examined, but no difference was noticed. Attempts were also made to find out whether there was any deposition of fatty substances on the walls of the tracheides in the older leaves which interfered with their activity. Various microchemical tests were made, and the tracheides from leaves of different ages were separated and were put in solutions of Sudan III and scarlet R, but no obvious absorption of dye was observed.

DISCUSSION.

It is evident from the above that the functional activities of the cells of the mesophyll do not terminate abruptly, but they show signs of decay one by one. Even those leaves which appeared quite green and healthy were, on microscopical examination, seen to possess in the intravascular regions cells which had lost their functional activities. It was recently pointed out by Briggs (2) that the photosynthetic activity of a leaf depends upon some internal factor which increases in proportion as the leaf becomes more and more mature, and the internal factor he thought to be the reactive chloroplast surface, which is not necessarily identical with actual chloroplast surface, an increase in the reactive surface bringing about a corresponding increase in the photosynthetic activity. To this statement of Briggs it could be added that the fall in the photosynthetic activity of a leaf also depends upon the decrease in the reactive chloroplast surface caused by the decay of the cells, and it decreases rapidly as the leaf becomes older till it stops entirely.

The question arises, Why do these photosynthetic elements become functionless and their chloroplasts disintegrate? It was stated before that the cells of the margin and apex, and in the meshes of the reticulate network which are situated farthest from the vascular tracheides, are the first to show the signs of decay. The formation of absciss layers would not be the cause of their decay by interrupting the water-supply, since, as it was pointed out, the decay of the cells begins very much before the formation of absciss layers, and in many cases the latter are not formed at all. Absciss layers are concerned with the fall of leaves rather than their decay. It would be possible to suppose that the cessation of functional activity at a definite period is a fixed hereditary character of the cells, but if that were the case one would expect the cells of a leaf to cease their activity simultaneously, and not in the regular sequence already described. There is no evidence to show that the apical, marginal, and intravascular cells, which are the first to decay, are also the first to be photosynthetically active.

The characteristic manner in which the photosynthetic elements show signs of decay favours the view that the water-supply has something to do with it, and it is probable that the tracheides, beginning with the smaller and followed by the larger, fail to perform their normal function of conduction. So the supply of water becomes inadequate and cannot meet the demand made by the cells for purposes of photosynthesis and transpiration. And the cells which are most distant from these tracheides, and so unfavourably placed, would be the first to suffer from this shortage of water-supply, as all the water would be utilized by the cells in the immediate vicinity of the tracheides. The water-conducting elements at the margins of a leaf might be thrown out of function much earlier than those placed centrally, because transpiration is more vigorous from the marginal areas of a leaf than from the central portions, which would make the tracheides inactive sooner than the central ones. It was also noticed that the leaves of many plants showed signs of decay on the marginal areas though the central portions were quite green when a period of drought set in. This also can be attributed to the marginal areas transpiring more vigorously than the middle ones and to the inability of the marginal tracheides to cope with the increased demand for water.

But the experiments performed with the solutions of eosin did not support the view that the tracheides had failed to conduct water, as in older leaves the tracheides occupying the starchless areas had taken up the stain, though very faintly. The faint staining of these tracheides indicated that they had not lost the power of conduction of water, but that the cells which derived their supply of water from them were dead and very little water was absorbed by them. Consequently a very minute quantity of the solution of the dye reached them and feebly coloured them. But in the case of veins occupying the living areas the cells continued to absorb water (and not the dye) from the tracheides, so that the dye solution became concentrated in the tracheides and stained them very conspicuously. So it was not the tracheides that were functionless but the mesophyll cells that were dead.

Microscopical investigation corroborated this view, as no change in structure or in chemical composition of the walls of the tracheides was detected which would account for their functional decay. This view of the functional decay of the tracheides in the older leaves would also be untenable in the light of the cohesion theory of transpiration advanced by Dixon (3), who assigned a purely mechanical function to these conducting elements.

This curious phenomenon could, however, be explained on two main suppositions. Firstly, as a leaf begins to mature, its demand for water also increases on account of the increased rates of photosynthesis and transpiration. And the tracheides, though continuing to perform their normal function, could not conduct the required amount of water, as the specific conductivity (per unit area) of these tracheides will remain the same after they have once assumed their permanent form. So the cells situated farthest from the terminal tracheides would be the first to suffer from the shortage of water, as all the water conducted by the tracheides would be utilized by the neighbouring cells.

But this idea would suggest that when some of the cells are dead a condition of equilibrium between the demand and supply of water is reached. As a result, the photosynthetic decay should not proceed further, and the leaf should continue to remain functional during the life of the plant if some other factor does not operate in causing its decay. But this conclusion could be avoided on the second supposition.

It was observed that the leaves with dead cells on the margin and in the intravascular regions remained functional for a very long time, when probably the supposed condition of equilibrium had been reached, but as growth continued towards the apex of the shoot and new leaves unfolded and matured, the condition of equilibrium in the old leaf was disturbed, because the total demand for water increased and the pull exerted on water in the woody axis increased, though it remained the same in the case of the older leaf. This would again bring about shortage of water-supply, causing the decay of some more photosynthetic elements unfavourably situated with regard to the supply of water. This further increase in the number of dead cells would again reduce the pull exerted on water by the old leaf. In this way the functional activity of a leaf will be brought to a standstill. It is possible that a condition of equilibrium between the demand and supply of water is obtained many times before the photosynthetic activity of a leaf entirely ceases, and the decay of the leaf is delayed or hastened by the influence of other internal or external factors, such as fluctuations in the rate of growth, light, temperature, and humidity of the air. And it may be due to the very same causes, as it was observed that during the wet months the number of leaves showing the characteristic decay found on various plants was very much less than the number of such leaves in hot weather.

The form of gradual decay described in the plants mentioned above may not be visible externally in the leaves of some plants on account of their thick texture, the nature of the vascular network, and many other causes, but it is maintained that here also the decay proceeds internally in the manner described owing to shortage of water, and commencing in the cells situated at the greatest distance from the source of water-supply.

Willstätter and Stoll (13) failed to obtain constant values of the assimilation numbers

$$\left(\text{assimilation number} = \frac{\text{amount of } CO_2 \text{ assimilation in 1 hour}}{\text{chlorophyll content}}\right)$$

of leaves of the same species in different stages of development or at different times in spring and autumn. Their tables show that after a time, although the chlorophyll content increases, yet the assimilation number diminishes, which, in their opinion, is due to some internal factor (enzyme) limiting the rate of assimilation. But on closer examination of their results it is seen that, though the chlorophyll content increases, the water content remains nearly the same or decreases. This decrease in the water content may be the chief internal factor limiting the rate of assimilation and may be the cause of the variable values of the assimilation numbers. In the accompanying tables are shown the amounts of water content, the chlorophyll content, the carbon dioxide assimilation per hour per sq. cm. of the leaf surface, and the assimilation numbers, as calculated from their results.

TABLE I.

(From the data of Wilstätter and Stoll (13) on 'Assimilation numbers of leaves from the same plant but in different stages of development'.)

Species.	*Age of the Leaf.*	*Water Content in grm.*	*Chlorophyll Content in grm.*	*CO_2 Assimilation in grm.*	*Assimilation Number.*
Acer pseudo-platanus	Young leaves	0·010	0·000014	0·00016	11·8
,, ,,	Old leaves	0·008	0·000051	0·00026	5·2
Tilia	Young leaves	0·014	0·000012	0·00017	14·2
,,	Old leaves	0·010	0·000042	0·00024	6·6

TABLE II.

(From the data of Willstätter and Stoll (13) on 'Assimilation numbers of leaves of the same species at different times in spring'.)

Species.	*Date.*	*Water Content in grm.*	*Chlorophyll Content in grm.*	*CO_2 Assimilation in grm.*	*Assimilation Number.*
Aesculus hippocastanum	29th April	0·029	0·000038	0.00042	11·1
,, ,,	7th May	0·030	0·000030	0·00036	12·1
,, ,,	3rd June	0·017	0·000051	0·00032	6·4
Tilia	4th May	0·013	0·000014	0·00015	10·6
,,	14th May	0·010	0·000015	0·00023	16·0
,,	5th June	0·009	0·000041	0·00029	7·1

The irregularities in their results, such as are found in autumn leaves and in normal leaves of various plants, could also be explained by the fact that it would be very difficult to select leaves in the same stages of photosynthetic activity for experimentation, as leaves of apparently the same age or at the same distance from the apices would not be in the same stage of photosynthetic activity, and a leaf which on external examination would appear quite fresh and healthy might be in an advanced stage of photosynthetic decay.

CONCLUSION.

It is clear from the above investigation that photosynthesis is dependent upon water content, and under normal conditions it is inadequate water-supply which terminates the photosynthetic activity of leaves and ultimately causes their decay.

SUMMARY.

1. In nature, the decline in the photosynthetic activity of leaves is due to shortage of water-supply, and the photosynthetic elements placed most unfavourably are the first to lose their power of assimilation. The photosynthetic decay first appears in the marginal and intravascular regions and then gradually proceeds inwards towards the centre and the main veins.

2. The shortage of water is not caused by the failure of the water-conducting elements to perform their function.

3. The shortage of water is caused in two ways. (i) As a leaf matures and increases in surface area, its demand for water also increases, owing to the increased rates of photosynthesis and transpiration, but the specific conductivity of the tracheides remains the same after they have gained their permanent form. This causes a shortage of water, and the cells most distant from the veins lose their activity. (ii) As more leaves unfold towards the apex, the pull exerted by the living cells of the upper leaves on water in the woody axis increases, while in the old leaf it remains the same. This causes a further shortage of water.

4. The relations between the demand and supply of water can be influenced by external and internal factors, and they may affect the functional duration of the leaves.

5. It has been explained why Willstätter and Stoll failed to obtain constant values of the assimilation numbers of the leaves of the same species in different stages of development or at different times in spring and autumn, and also in normal leaves of different plants.

I have to thank Prof. W. Stiles for suggestions and references to literature during the course of this investigation.

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NOTES.

FASCIATED ROOTS OF CALTHA PALUSTRIS, L.—Although the root of *Caltha palustris*, L., has been extensively examined in many laboratories throughout Europe and America, there has been no report, as far as the writer is aware, of fasciation in this organ. In fact, roots in general are said to exhibit fasciations less frequently than do other organs of plants.

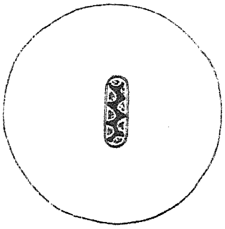

FIG. 1.

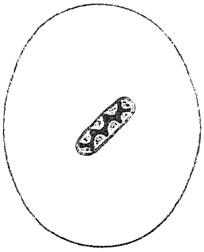

FIG. 2.

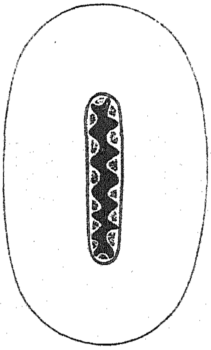

FIG. 3.

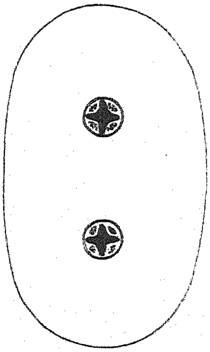

FIG. 4.

An examination of roots of *Caltha palustris* collected near Edmonton, Alberta, in the spring of 1923, revealed some rather striking departures from the typical structure. Approximately two-thirds of the roots in the collection showed a tetrarch stele, most of the remainder were pentarch, and a small number hexarch. Four specimens differed quite markedly from the normal in the structure of their vascular tissues. These specimens were root segments varying in length from 4 to 10 cm. Two of them were abnormally large and considerably flattened (Figs. 3, 4), the other two being somewhat above the average in size and almost circular in transverse section (Figs. 1, 2). In each case the stelar structure was constant throughout the

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length of the segment. Also, in each, several lateral roots arose in the normal way from the pericycle region.

The gross anatomy of these abnormal roots is shown in transverse section in the accompanying diagrams. In three of the roots the vascular tissue is band-shaped (fasciated) and consists of an unusually large number of protoxylem and phloem groups (Figs. 1, 2, 3). It will be noted that in the broadest of these steles there are no fewer than fourteen protoxylem groups and a corresponding number of phloem groups. A camera lucida drawing (Fig. 5) of a part of a stele (Fig. 1) shows normal pericyclic and endodermal layers and a very regular arrangement in the flattened stele of protoxylem and phloem groups. The fourth specimen (Fig. 4) possesses two steles, each enclosed by an endodermis and resembling in detail the stele of a typical tetrarch root.

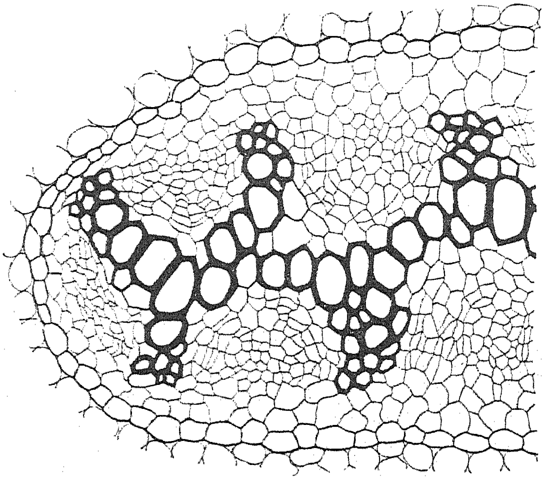

FIG. 5.

It is natural that one should speculate upon the causes of fasciation and distely as exhibited in these roots. A fasciation is said to arise in one of two ways, (*a*) by the fusion of several axes ordinarily distinct, (*b*) by the lateral expansion of the meristematic apical cone of an axis. The *Caltha* roots appear to be of the latter type, since they show the following features: outline smooth and without grooves or furrows, endodermis continuous and regular (Figs. 1, 2, 3), steles symmetrically placed and oriented (Fig. 4). It is probable that in the distelic root the steles arose from two plerome primordia embedded in a common periblem, whereas in the other cases an unusually large number of protoxylem and phloem groups were differentiated from a flattened plerome.

Church's concept of 'growth-centres', as quoted by Worsdell,[1] is of interest here. 'In the "fasciated" system the centric distribution around a point (the single growth-centre) is changed for an attempt at similar distribution around a number of centres . . . or around a longer or shorter series of such points constituting a line, with the result that great disturbances ensue owing to the impossibility of normal uniform growth expansion in such a system.' In these abnormal *Caltha* roots, however, it can scarcely be maintained that departure from centric distribution around a single growth-centre has been accompanied by 'great disturbances', such as ordinarily characterize fasciation phenomena.

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[1] Worsdell, W. C.: The Principles of Plant Teratology, vol. i, p. 87. The Ray Society, London, 1915.

In regard to the physiological cause of fasciation in these roots a suggestion made by various investigators of fasciation phenomena is adopted. It is probable that, at certain critical stages in the development of the root primordia, superabundant nutrition induced the formation of abnormal growth-centres. Lopriore and other investigators have produced fasciation in roots of bean seedlings by amputating the tip of the radicle, and in stems by excising the main axis of the plumule. Brannon [1] describes fasciated shoots obtained from stumps of cottonwoods and willows. It has been shown by Reed [2] that wounding may divert food supplies so as to produce superabundant nutrition in certain parts and a consequent abnormal growth of those parts. That wounding was the immediate cause of fasciation in *Caltha* roots is scarcely probable, in view of the symmetry constant throughout considerable lengths. Nor can flattening of the stele be ascribed to external pressure, because, although the stele is flattened, the root as a whole is not necessarily so (Figs. 1, 2). Worsdell's suggestion that fasciation is probably a pathological condition can scarcely be accepted in the case of these *Caltha* roots. According to Worsdell (loc. cit., p. 95), a pathological condition is 'stimulated to appear by the presence of superabundant nutrition, which produces a subtle diseased condition, thus giving rise to a hypertrophied growth which destroys the balance of the organism'. Judging by the appearance of the roots of *Caltha* the balance of the organism has not been destroyed however; although presenting unusual structural features these roots appear to be physiologically normal and well balanced.

E. H. MOSS.

BOTANICAL LABORATORIES,
UNIVERSITY OF ALBERTA,
EDMONTON, CANADA.

THE DRY-NEEDLE METHOD OF MAKING MONOSPOROUS CULTURES OF HYMENOMYCETES AND OTHER FUNGI.—Students of mycology and phytopathology frequently find it necessary to cultivate certain species of fungi from single spores. The procedure employed for isolating and germinating the individual spores should permit of the work being performed simply, rapidly, and with perfect accuracy.

The poured-plate method, used so generally by bacteriologists, has often been employed in mycological investigations. While satisfactory in some respects, it falls far short of perfection; for it is slow and cumbersome, and if the medium used for germinating the spores is not perfectly clear there is always the possibility, when the transfer is being made from the Petri dish, that the mycelium taken up may have been derived from more than one spore. Furthermore, if one requires to sow a spore of particular size or shape, the poured-plate method is of little or no value.

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[1] Brannon, M. A.: Fasciation. Bot. Gaz., lviii. 518–26, 1914.

[2] Reed, T.: Some Points in the Morphology and Physiology of Fasciated Seedlings. Ann. Bot., xxvi. 389–402, 1912.

Edgerton[1] has described a method of isolating a particular spore from a liquid medium by means of a small capillary tube suitably attached to the substage of the microscope. The upper end of the tube is sealed, while the lower end is drawn out to a fine point. This point is lowered carefully until it comes in contact with the spore, whereupon a small drop of ether is placed on the upper closed end of the tube, thus causing the spore to be sucked up into the tube's interior. By gently heating the tube with a small flame, the spore, together with the liquid in which it is enveloped, is then driven from the tube and deposited upon the medium on which it is to germinate. Recently, Roberts[2] has suggested certain improvements which might be made to this apparatus, particularly in respect to the adjustment of the capillary tube to the substage.

While working with certain species of the genus *Coprinus*, the following method of making monosporous cultures from particular spores was devised. It is simple and accurate, and yet permits of the cultures being made very rapidly. No apparatus is required, other than that which is to be found in every mycological laboratory.

If a sterilized glass slide is placed under a fruit-body, such as that of *Coprinus sterquilinus*, which is rapidly shedding spores, a suitable spore-deposit may be obtained in from one to two minutes. An examination of the slide under the microscope will show that the spores are well separated from one another, thus making the selection of individual spores a comparatively simple matter. The slide should be kept in a sterile Petri dish until required. If spore-deposits of this kind have not been procured, and fruit-bodies are not available, a relatively thicker spore-deposit which has been taken on a glass slide for ordinary cultural purposes may serve equally well, as certain spots in it will generally be found where the spores are sufficiently separated from one another.

A number of Petri dishes should be fitted up with ring-cells and cover-slips (Fig. 1), as described by Duggar.[3] A large Petri dish, 14 cm. in diameter and 2 cm. high, has been found to be very suitable for this work. In the bottom of the dish is placed a sheet of filter-paper, in which ten circular holes have been cut. In each hole a ring-cell, 17 mm. in diameter and 10 mm. high, is inserted, and cover slips (without vaseline) are placed on the tops of the ring-cells. The whole is then sterilized in hot air. When cool, a little water is poured over the filter-paper, and a drop of the germinating medium is touched to the bottom of each cover-slip. A Petri dish fitted up in the manner described is shown in section, in Fig. 1, A, and in surface view in Fig. 1, B.

When single spores are to be isolated, a slide bearing a spore-deposit is placed under the low power of the microscope, and moved about until a spore of the required shape or size is brought into the centre of the field. Then, holding a fine sewing-needle, such as is shown in Fig. 1, C, between the thumb and forefinger of the right hand, the point is lowered slowly downwards until it comes into contact with the desired spore. When touched, the spore leaves the glass slide and adheres readily to the needle-point. Fig. 2 represents a small portion of a glass slide bearing spores of *Coprinus sterquilinus* sufficiently separated from one another to permit of any one of them being easily picked up without danger of touching any of its fellows. The needle-point shown in this figure is drawn on the same scale as the spores, and has a single spore adhering to its point. When the spore is seen to be separated from the slide and to be attached securely to the needle-point, it is transferred to the ger-

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[1] Edgerton, C. W. (1914): A Method of picking up Single Spores. Phytopathology, vol. iv, No. 2, pp. 115–17.

[2] Roberts, J. W. (1923): A Method of isolating Single Spores. Ibid., vol. xiii, No. 12, pp. 558–60.

[3] Duggar, B. M. (1909): Fungus Diseases of Plants, New York, p. 95.

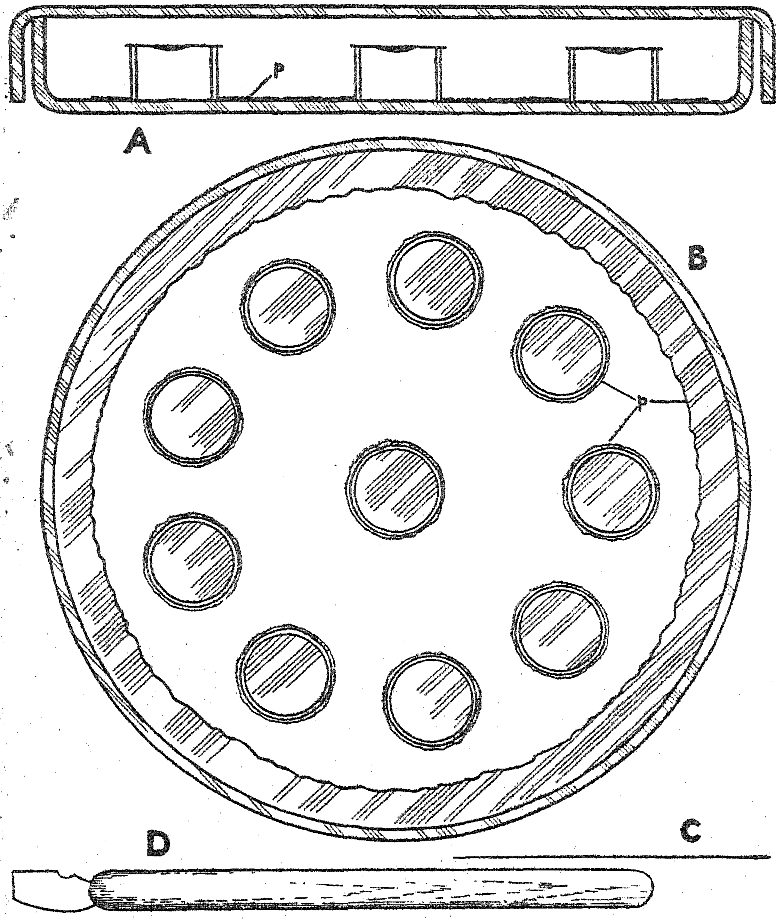

FIG. 1. A and B, a Petri dish shown in section and in surface view respectively. The ring-cells are set in holes in a sheet of wet filter-paper, *p*. The cover-slips in A bear hanging drops of agar. C, a needle used for making monosporous cultures. D, an agar spade, used for transferring hanging drops containing mycelia from ring-cells to poured plates. A, B, and D, reduced to 2/3; C, natural size.

minating medium simply by touching the end of the needle to the drop on the lower side of one of the cover-slips. By examining the drop under the microscope, the spore is quickly located; and thus a final and absolute proof is obtained that the particular spore selected on the slide, and no other, has been transferred to the drop. With a little practice, spores may be picked up very rapidly. With *Coprinus sterquilinus*, for example, ten monosporous cultures were made without difficulty in ten minutes.

Later on, when germination has taken place, the mycelium is removed from the cover-slip to a plate of sterile agar or gelatine. A platinum loop may be employed in making this transfer, but the best results have been obtained by using a piece of safety-razor blade about 1 cm. wide, placed in the end of a wooden handle, as shown in Fig. 1, D. With this little instrument, which may be called an *agar spade*, the young mycelium can be removed from the cover-slip without injury. I have found by experience that the use of the agar spade in the manner just described greatly increases the chance of making transfers successfully.

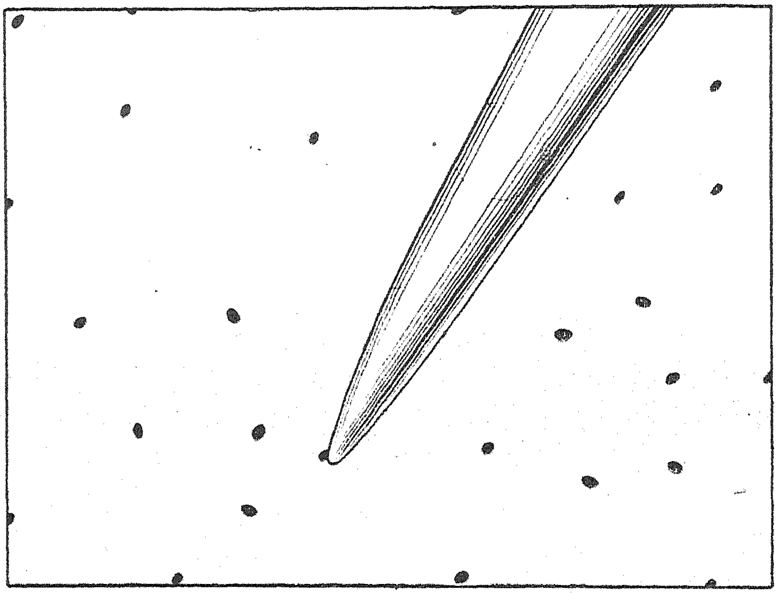

FIG. 2. Piece of a glass slide with a dry spore-deposit of *Coprinus sterquilinus*. In the middle, a dry needle-point used to pick up individual spores. A spore is attached to its tip. Magnification, 107.

The adhesion of the spore to the needle-point does not seem to be an ordinary electrical phenomenon, as spores deposited on a thin brass plate were picked up without difficulty, although both plate and needle were grounded by means of fine copper wires. The amount of moisture in the atmosphere also seems to be of no consequence, as spores could be picked up equally well in a dry or saturated atmosphere. Spores seem to behave exactly like small particles of glass, wool, or gelatin; for these, when touched, also attach themselves readily to the needle-point. This property of adhering to other objects, with which very small objects are endowed, in all probability is due to the fact that very small objects possess a very large surface relatively to their mass, in consequence of which the force of adhesion becomes of great importance relatively to the force of gravitation. In some instances the spores could be moved about on the slide without becoming readily attached to the needle-point; but, even under these conditions, it was always found possible to pick up any required spore after a few trials.

Cultures of a number of species have been successfully made with the dry-needle method. Of these species the following may be mentioned:

Coprinus sterquilinus
,, *stercorarius*
,, *lagopus*
,, *curtus*
,, *ephemerus*
,, *niveus*

Coprinus cordisporous
,, *atramentarius*
,, *Rostrupianus*
Panaeolus campanulatus
Stropharia semiglobata
Bolbitius, sp.

While all of these species belong to the Hymenomycetes, there is every reason to believe that the dry-needle method might be employed with equal success in dealing with other groups of the Basidiomycetes, as well as with many of the Ascomycetes.

SUMMARY.

A dry-needle method of making monosporous cultures of Hymenomycetes and other fungi has been described. The method is at once very simple, very rapid in its application, and extremely precise. Relatively to the poured-plate method, when it is necessary to make numerous cultures, it effects a considerable saving of time and energy and gives reliable results.

The dry-needle method was devised during the course of certain mycological researches carried out in the Department of Botany at the University of Manitoba. The work was made possible by a research scholarship granted by the Canadian Society of Technical Agriculturists. The writer wishes to express his indebtedness to Professor A. H. R. Buller for his valuable suggestions and stimulating criticism.

W. F. HANNA.

STOMATA AND PHYLOGENY.—In Hutchinson's last paper of his series on the Phylogenetic Classification of Flowering Plants (Kew Bull., 1924, pp. 114–35) he calls attention to the fact that many arborescent families, in contrast to herbaceous ones, are characterized by having stomata with subsidiary cells parallel to the pore. To quote (p. 119):

'It is significant, however, that many very natural families are either entirely woody or entirely herbaceous. Examples of the former are *Magnoliaceae*, *Anonaceae*, and *Lauraceae*, &c.; of the latter, *Ranunculaceae*, *Papaveraceae*, and *Cruciferae*. And in the rather primitive groups, at any rate, the difference in habit is accompanied by a marked difference in the structure of the stomata; in the woody group the guard-cells are usually accompanied by special subsidiary cells parallel to the pore, whilst in the early herbaceous groups the guard-cells have no special subsidiary cells. It is difficult to suggest just what the significance of this different type of stoma represents. Of course in more advanced groups the two types are often found in the same family the elements of which are brought together perhaps by parallel evolution. It seems significant that large natural families such as the ligneous *Rubiaceae* should have a characteristic type of stoma.'

The writer of this note has independently been struck with this type of stoma from the phylogenetic point of view, and in his paper on the Strobilus Theory of Angiospermous Descent, submitted to the Linnean Society of London in November 1922, he offered some remarks on the subject. The paper appeared in abstract only (see Proc. Linn. Soc., 1923, pp. 51–65), and the part relating to stomata was omitted. With the kind permission of the Council of the Society, this paragraph is herewith reproduced from the original manuscript. In the light of Hutchinson's observations, set forth above, it may be of some interest to botanists.

'A minor point connected with stomata is perhaps here worthy of notice, as it favours the Bennettitean part of the Strobilus Theory. Thomas and Bancroft, in their paper on the cuticles of Cycadean Fronds [Trans. Linn. Soc., 1913, viii. 155–204], show that the Bennettitales possessed a pair of subsidiary cells parallel to the guard cells of the stoma, which they think may have been cut off from the original stomatal mother-cell. In the Nilssoniales and the recent Cycads they find subsidiary cells of a different character; six or more in number occur, more or less radially arranged round the guard cell. They point out that the Gnetales have a type of subsidiary cell similar to that of the Bennettitales; but they do not institute any comparison with the Angiosperms. This, however, is interesting, for the Magnoliaceae and allied families have a pair of subsidiary cells parallel to the guard cells or pore of the stoma, as it is sometimes expressed. Such a feature in common between the Magnoliaceae and the Bennettitales would be trivial, if it stood alone; but taken along with the other points of agreement it may have significance. At any rate, within the Angiosperms themselves this kind of subsidiary cell, sometimes called the Rubiaceous type, promises to be of some value in the tracing of affinities. But a word of warning is necessary. The development of these subsidiary cells must be followed in each case to see whether they be true or false. Ordinary epidermal cells may in some cases simulate true subsidiary cells by arranging themselves parallel to the pore.'

J. PARKIN.

BLAITHWAITE, WIGTON, CUMBERLAND
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